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TECHNICAL PAPER No. 1

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THE REMOVAL OF SODIUM CARBONATE  
FROM SOILS

BY

WALTER P. KELLEY AND EDWARD E. THOMAS



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THE REMOVAL OF SODIUM CARBONATE  
FROM SOILS\*

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WALTER P. KELLEY AND EDWARD E. THOMAS

It is well known that alkali soils which contain sodium carbonate are especially difficult to reclaim. The removal of the excess of soluble salts by leaching is greatly hindered by the deflocculated condition of such soils. The deflocculation increases as the concentration of electrolytes decreases. Consequently after partial leaching the soil may become practically impervious to the penetration of water. The deflocculated condition, however, is by no means the only difficulty to be overcome in the reclamation of the so-called "black-alkali" soils. Perhaps still more important is the fact, as shown in a previous paper,<sup>7</sup> that injurious concentrations of OH-ions may remain in the soil after practically all of the neutral salts have been removed. As a matter of fact, the deflocculated condition of such soils, and also that of certain leached saline soils, previously noted by Scofield and Headley<sup>16</sup> and by Sharp,<sup>18</sup> is probably due primarily to the chemical alkalinity of the soil solution.

Cameron and Patten<sup>2</sup> showed that with certain soils it is possible to leach out practically all of the soluble salts, including sodium carbonate; but as will be discussed more fully in a separate paper by Cummins and Kelley,<sup>4</sup> certain other constituents may be present which are not sufficiently soluble to be removed by ordinary leaching, but which impart injurious alkalinity to the soil solution. The result is that the soil may still be toxic to plants after the principal part of the soluble salts has been removed.

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\* Paper No. 90, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.



That black-alkali soils may be improved by special treatment has long been recognized. Early in his investigations on alkali soils, Hilgard recommended the application of gypsum as a means of converting sodium carbonate into calcium carbonate and sodium sulfate. Many years later, Loughridge<sup>14</sup> pointed out that it is necessary to apply approximately twice as much gypsum as is indicated by the analysis of a water extract of the soil. The conclusion of Loughridge is in harmony with recent investigations of this laboratory<sup>7</sup> which show that the total alkalinity is not measured accurately by the prevailing methods of analysis.

Cameron and Seidell<sup>3</sup> and Breazeale<sup>1</sup> concluded that it is not possible to precipitate the carbonate completely, by means of gypsum, where the soil also contains a high concentration of neutral salts. They showed that when equilibrium has been established following the application of gypsum, the concentration of  $\text{CO}_3$  may still remain relatively high.\*

Lipman and Gericke<sup>11</sup> employed manure, Hibbard<sup>6</sup> used manure and other organic materials, and Lipman and Sharp<sup>10</sup> applied sulfuric acid in the treatment of certain black-alkali soils of California. As an outgrowth of his work on the process of sulfonation, J. G. Lipman<sup>12</sup> suggested that the biological oxidation of elemental sulfur might prove to be an efficient means of decomposing sodium carbonate in soils. Recently Hibbard<sup>5</sup> published data which indicate that the application of elemental sulfur may prove to be a practical treatment for black-alkali soils.

In our investigation on the alkali problem of California special attention has been given to the black-alkali soils. Extended laboratory studies are being made on different phases of this subject. A carefully conducted field experiment on a tile-drained area near Fresno, California, is also being made. The results already obtained indicate that it is impracticable to reclaim certain portions of this area by the ordinary leaching process. We have been able to remove the greater part of the neutral salts by flooding the land, but toxic concentrations of sodium carbonate still remain in the soil. Certain plots have also been treated with gypsum followed by heavy flooding. An additional area has recently been treated with elemental sulfur. In connection with these experiments a laboratory study has been made on the effects

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\* The equilibrium between carbonates and neutral salt solutions is being studied further in this laboratory.



of various neutralizing substances, the results of which are presented in this paper.

Five soils have been used in these studies. No. 905 is a fine sandy soil from Riverside, California; 909 is a fine sandy loam from Farmersville, California; 2736 is a clay loam from Salt Lake City, Utah; and 2753 and 2754 are fine sandy loams from Fresno, California. Soil 2754 was taken from the area referred to above, before it was treated with sulfur. Each sample was taken from locations where crops had failed.

TABLE 1  
SOLUBLE CONSTITUENTS OF SOILS

	(Parts per million)				
Laboratory number	905	909	2736	2753	2754
CO <sub>3</sub> .....	210	255	810	1200	420
HCO <sub>3</sub> .....	610	945	656	1281	778
Cl.....	2600	124	4468	1796	1840
SO <sub>4</sub> .....	3555	108	1963	482	788
NO <sub>3</sub> .....	289	265	155	750	419
PO <sub>4</sub> .....	4	23	57	138	73
SiO <sub>2</sub> .....	28	66	75	59	55
Ca.....	5	2	5	7	7
Mg.....	86	12	28	97	22
K.....	336	52	60	52	30
Na.....	3688	644	4745	2860	2370
Total salts.....	12000	2150	12380	9425	6415
pH-value.....	9.4	9.6	9.8+	9.8+	9.8+

#### COMPOSITION OF THE SOILS

The analysis of water extracts of these soils (1:5) is recorded in table 1. It will be noted that the soluble constituents varied considerably, both quantitatively and qualitatively. The total salt content of soils 905 and 2736 was high, that of soils 2753 and 2754 intermediate, and that of soil 909 comparatively low. The content of soluble carbonate, which is of special interest in this connection, also varied considerably, ranging from 210 parts per million in soil 905 to 1200 parts per million in soil 2753. A very large proportion of the soluble salts present was composed of sodium compounds.

Total CO<sub>2</sub> was determined by gently heating samples of these soils with 4 per cent HCl and aerating the CO<sub>2</sub> into KOH-bulbs. The CO<sub>2</sub> equivalent of the soluble CO<sub>3</sub> and HCO<sub>3</sub> was subtracted from the total CO<sub>2</sub> and the remainder calculated as CaCO<sub>3</sub>.



## CALCIUM CARBONATE CONTENT OF SOILS

(Per cent)

Laboratory number	905	909	2736	2753	2754
CaCO <sub>3</sub> .....	2.82	0.60	8.55	0.20	0.16

The results show that soils 2736 and 905 each contain rather high percentages of CaCO<sub>3</sub>, while soil 909 contains a considerable amount and soils 2753 and 2754 much smaller amounts.

## SULFOFICATION EXPERIMENTS

Three different quantities of elemental sulfur were added to equal portions of each soil. After mixing thoroughly, distilled water was added in amounts sufficient to bring the moisture content to the optimum. The several portions were placed in fruit jars and kept at laboratory temperature. After standing for certain periods of time, a part of the soil was withdrawn from each jar and the water-soluble CO<sub>3</sub>, HCO<sub>3</sub>, SO<sub>4</sub> and Ca, and the pH-values were determined. The average results of duplicate experiments are submitted in tables 2, 3, 4, 5 and 6.

TABLE 2

SULFOFICATION DATA, SOIL 905

(Parts per million)

Sulfur added	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 2 weeks						
200	180	678	3589	2	9.3	6
400	150	724	3724	4	9.3	14
800	142	648	3904	10	9.3	15
After 4 weeks						
200	127	610	3951	8	9.0	66
400	105	579	4052	20	8.9	41
800	52	648	4717	42	8.8	48
After 6 weeks						
200	135	533	4147	27	9.0	99
400	97	533	4458	40	8.9	75
800	30	541	5299	75	8.6	73
After 9 weeks						
200	112	533	3828	7	9.0	45
400	75	577	4356	42	8.8	67
800	37	457	5249	100	8.6	71
After 15 weeks						
200	112	404	4134	35	9.2	96
400	60	396	4534	60	8.9	82
800	45	373	5788	132	8.6	93



TABLE 3  
SULFOFICATION DATA, SOIL 909  
(Parts per million)

Sulfur added	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 2 weeks						
400	157	861	234	2	9.1	10
800	165	839	330	2	9.2	9
1600	142	816	471	2	9.2	8
After 4 weeks						
400	165	823	371	2	9.2	22
800	142	724	531	2	9.0	18
1600	90	686	797	2	8.7	14
After 6 weeks						
400	135	777	452	2	9.3	29
800	120	632	791	2	8.9	28
1600	15	587	1381	2	8.4	27
After 9 weeks						
400	60	709	860	2	8.6	63
800	15	518	1752	2	8.5	68
1600	0	351	3265	287	7.8	66
After 15 weeks						
400	30	610	1013	6	8.5	75
800	0	434	1897	64	7.5	74
1600	0	290	3730	497	7.3	75

TABLE 4  
SULFOFICATION DATA, SOIL 2736  
(Parts per million)

Sulfur added	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 2 weeks						
1600	810	701	1981	2	9.4	0
3200	862	594	2024	2	9.4	1
6400	645	854	2064	2	9.2	1
After 4 weeks						
1600	412	953	2708	2	9.4	16
3200	315	945	2856	2	9.4	9
6400	165	884	3346	2	9.3	7
After 6 weeks						
1600	217	640	3232	2	9.4	26
3200	105	473	3644	2	9.2	18
6400	15	434	4456	2	8.5	13
After 9 weeks						
1600	105	388	4140	2	8.9	45
3200	0	381	4662	41	7.2	28
6400	0	328	5391	195	8.0	18



TABLE 4—(Continued)

Sulfur added	CO <sub>2</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 15 weeks						
1600	45	327	4196	27	8.9	47
3200	0	351	4976	219	7.1	31
6400	0	381	5891	486	7.6	20

TABLE 5  
SULFOFICATION DATA, SOIL, 2753  
(Parts per million)

Sulfur added	CO <sub>2</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 2 weeks						
1600	1020	1936	705	5	9.6	5
3200	1012	1624	853	10	9.6	4
6400	915	1944	994	4	9.6	3
After 4 weeks						
1600	495	1950	1547	2	9.3	22
3200	442	1273	2458	2	9.3	21
6400	135	693	3855	2	9.0	18
After 6 weeks						
1600	307	1342	2597	5	9.5	44
3200	157	655	3990	2	9.2	37
6400	37	595	4621	2	8.6	22
After 9 weeks						
1600	250	778	3447	3	9.3	62
3200	30	662	4715	11	8.5	44
6400	0	572	5178	60	8.0	24
After 15 weeks						
1600	97	519	3955	7	8.9	72
3200	0	427	4934	66	7.5	46
6400	0	274	6596	457	7.1	32

TABLE 6  
SULFOFICATION DATA, SOIL 2754  
(Parts per million)

Sulfur added	CO <sub>2</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 2 weeks						
400	225	892	958	2	9.6	14
800	172	778	1388	2	9.3	25
1600	90	747	1759	2	9.1	20
After 4 weeks						
400	172	671	1547	2	9.1	63
800	60	556	2105	2	8.7	55
1600	15	465	2724	2	8.4	40



TABLE 6—(Continued)

Sulfur added	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	Ca	pH	Sulfur oxidized (per cent)
After 6 weeks						
400	75	701	1734	2	8.8	79
800	0	533	2438	2	7.6	69
1600	0	388	3190	70	7.4	50
After 9 weeks						
400	120	557	1859	2	8.9	89
800	0	510	2675	5	8.2	79
1600	0	343	3439	157	7.2	55
After 15 weeks						
400	142	480	1955	2	8.9	97
800	0	457	2919	56	8.0	89
1600	0	305	4109	355	7.2	69

It will be noted that the sulfur underwent reasonably active oxidation in each soil. Within the first two weeks of the experiment, the soluble CO<sub>3</sub> decreased and the content of SO<sub>4</sub> increased considerably. At the close of the fourth week still further amounts of CO<sub>3</sub> had disappeared and the oxidation process continued throughout the fifteen weeks of the experiment. It is especially interesting that vigorous oxidation of sulfur took place in the presence of comparatively high concentrations of NaCl, Na<sub>2</sub>SO<sub>4</sub> and Na<sub>2</sub>CO<sub>3</sub>.

A relatively high percentage of the added sulfur was finally oxidized in one or more portions of each soil, except 2736. In the latter soil the rate of oxidation was considerably less rapid, possibly because of the relatively high concentration of chloride which it contained.\* With the exception of soil 905, the products resulting from the oxidation of the larger amounts of sulfur finally neutralized the last trace of soluble CO<sub>3</sub> and decomposed a considerable part of the HCO<sub>3</sub> also.

As shown by the pH-values, none of these soils became acid, although the reaction of each soil except 905 finally approached neutrality.

Except in the case of soil 905 almost no calcium was made soluble until practically all of the soluble CO<sub>3</sub> had disappeared. Later, how-

\* It is immaterial, so far as these experiments are concerned, whether biological agencies were solely responsible for the oxidation of the sulfur. (See MacIntire.<sup>15</sup>) The important point is that reasonably active oxidation took place. It is, of course, possible that still more active oxidation might have been induced by inoculating the soil or sulfur with specially active strains of certain species of sulfifying bacteria,<sup>13</sup> but the final effect on the soil would probably not have been materially different.



ever, the solubility of calcium was increased. The fact that small amounts of soluble  $\text{CO}_3$  still remained in soil 905 after substantial amounts of calcium had been dissolved was probably due to the combined effects of the large amounts of  $\text{CaCO}_3$  and neutral sodium salts, especially the sulfate, which this soil contained.

TABLE 7  
EFFECT OF  $\text{H}_2\text{SO}_4$  ON ALKALI SOILS  
(Parts per million)

$\text{H}_2\text{SO}_4$ added	$\text{CO}_3$	$\text{HCO}_3$	Ca	pH
Soil 905				
None	210	610	5	9.4
612	120	1037	100	9.0
1225	60	1403	250	8.6
1633	Trace	1769	302	8.3
2450	0	2348	562	8.2
4900	0	3355	1315	8.0
Soil 909				
None	255	945	2	9.6
612	30	1128	25	9.0
1225	Trace	1357	97	8.3
2450	0	1601	422	7.6
4900	0	1647	1175	7.2
Soil 2736				
None	810	656	5	9.8+
1225	240	1647	70	9.6
1633	6	2120	137	8.3
2450	0	2440	527	8.2
4900	0	2410	1340	7.4
Soil 2753				
None	1200	1281	7	9.8+
1225	210	2379	10	9.6
2205	Trace	2836	32	8.3
2450	0	2882	60	7.9
4900	0	1540	305	7.0
Soil 2754				
None	420	778	7	9.8+
612	105	1067	5	9.4
1102	Trace	1311	20	8.3
1225	0	1434	50	8.1
2450	0	1327	325	7.4
4900	0	152	805	5.8



## EXPERIMENTS WITH SULFURIC ACID

Portions of the same soils were treated with sulfuric acid solutions of various strengths. Two hundred grams of soil were shaken for one hour with 1000 cc. of the solutions. The solutions were then filtered and  $\text{CO}_3$ ,  $\text{HCO}_3$ , Ca and the pH-values were determined in the filtrates.

It will be seen (table 7) that the more dilute solutions of sulfuric acid decomposed only a part of the soluble carbonate, but that the stronger solutions removed the last trace of it. The bicarbonate determinations are especially interesting. They show that with each soil the normal carbonate was first converted into bicarbonate. As the strength of the acid was increased, the bicarbonate was also partially decomposed in soils 2753 and 2754. The fact that the greatest strength of acid used with soils 905, 909 and 2736 yielded extracts with the highest content of  $\text{HCO}_3$ , although  $\text{CO}_3$  disappeared when much weaker solutions were used, was probably due to the  $\text{CaCO}_3$  of these soils. This view derives support from the fact that with soils 2753 and 2754 the  $\text{HCO}_3$  did not increase materially after the soluble  $\text{CO}_3$  disappeared. The pH-values also indicate that  $\text{CaCO}_3$  was involved in these reactions.

The calcium determinations confirm the preceding statements. It will be noted that substantial amounts of calcium were dissolved in soils 905 and 2736 even before all of the soluble  $\text{CO}_3$  disappeared; when only a mere trace of  $\text{CO}_3$  remained in solution still greater amounts of calcium were dissolved in these soils and an appreciable amount in soil 909. Finally, large amounts of calcium were dissolved in each soil by the strongest solutions of sulfuric acid, but the amounts were much greater in soils 905, 909 and 2736 than in soils 2753 and 2754.

## EXPERIMENTS WITH CALCIUM SULFATE

The same soils were also treated with various amounts of calcium sulfate. After adding distilled water in the ratio of 1:5 and shaking for one hour, the extracts were analyzed. The results are shown in table 8.

It was found that as the amount of calcium sulfate was increased, a gradual reduction in the soluble  $\text{CO}_3$  and  $\text{HCO}_3$  took place in each



soil, but that the soluble  $\text{CO}_3$  was completely precipitated in only one soil (909) and in that case only when a large excess of calcium sulfate was added.

These results confirm the conclusion of Breazeale,<sup>1</sup> that it is not possible to precipitate the soluble  $\text{CO}_3$  completely by means of gypsum,

TABLE 8  
EFFECT OF GYPSUM ON ALKALI SOILS  
(Parts per million)

$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ added	$\text{CO}_3$	$\text{HCO}_3$	$\text{SO}_4$	Ca	pH
Soil 905					
None	210	610	3555	5	9.4
2150	135	351	5076	180	9.6
4300	105	259	6110	435	9.6
8600	60	290	8293	1105	9.4
17200	60	213	12256	2430	9.4
Soil 909					
None	255	945	108	2	9.6
2150	120	351	1334	20	9.5
4300	15	274	2402	170	8.4
8600	6	183	4544	822	8.3
17200	0	259	8817	2440	8.0
Soil 2736					
None	810	656	1963	5	9.8+
2150	690	351	3083	67	9.6
4300	555	320	4083	210	9.6
8600	195	183	6365	777	9.6
17200	120	213	10670	2410	9.6
34400	120	183	11948	2880	9.6
Soil 2753					
None	1200	1281	482	7	9.8+
2150	1185	961	1627	35	9.6
4300	1005	1006	2711	70	9.6
8600	510	945	4915	287	9.6
17200	135	839	9204	1800	9.6
34400	120	778	11705	2755	9.5
68800	120	671	11816	2800	9.4
Soil 2754					
None	420	778	788	7	9.8+
2150	330	335	1965	17	9.6
4300	240	351	3053	195	9.6
8600	165	381	5253	985	9.6
17200	135	335	9542	2620	9.4
34400	135	320	10218	2900	9.4



if the soil contains a high concentration of neutral sodium salts. In fact, sufficient amounts of sodium sulfate may be formed as a direct result of the treatment with gypsum, to prevent the complete precipitation of the carbonate.

Since the effect of elemental sulfur is dependent on its first being oxidized to sulfuric acid, and since the neutralizing effects of sulfuric acid and calcium sulfate are both directly proportional to their respective  $\text{SO}_4$  content, it is interesting to compare the effects of these materials on the basis of the  $\text{SO}_4$  actually added to or formed within the soil. Certain parts of the preceding determinations admit of fairly direct comparison on this basis. These show that whereas the smaller amounts of  $\text{H}_2\text{SO}_4$ , whether formed in the soil by the oxidation of sulfur or added as pure solutions, were approximately equally effective in removing normal carbonate, considerably greater amounts of the sulfur oxidation product were required to remove the last trace of it. On the other hand, when chemically equivalent quantities are considered, calcium sulfate proved to be decidedly inferior, both to elemental sulfur and to  $\text{H}_2\text{SO}_4$  solutions, at all stages of the process.

From the preceding discussion it is evident that the effect of oxidizing sulfur was somewhat different from that of sulfuric-acid solutions. With the former, the soil being kept at a moisture content approximating that of good tilth, there was a tendency for both sodium carbonate and sodium bicarbonate to be decomposed simultaneously.\* Except in the case of soil 905 the oxidation of sulfur did not materially increase the solubility of calcium until practically all of the sodium carbonate was decomposed. The sulfuric-acid solutions, on the other hand, caused a marked increase in  $\text{HCO}_3$ , and with certain of these soils calcium was dissolved before all of the soluble carbonate had disappeared.

In the sulfonation series a large part of the  $\text{CO}_2$  set free by the sulfur oxidation products probably escaped into the atmosphere, since the soil moisture must have become saturated with  $\text{CO}_2$  early in the experiment. With the use of sulfuric-acid solutions, on the other hand, normal carbonate was first converted into bicarbonate, just as takes place in the ordinary titration of carbonate solutions.

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\* Since this paper was written, Rudolfs published the results of an investigation on the effect of sulfur on two black-alkali soils. He found that with one soil normal carbonate was first converted into bicarbonate, whereas with the other soil the results were similar to our data. (*Soil Science*, vol. 13, no. 3, pp. 215-229.)



Since certain of these soils contained considerable amounts of  $\text{CaCO}_3$ , the decomposition of the bicarbonate did not set in until a considerable part of the  $\text{CaCO}_3$  had been converted into the bicarbonate also.

#### EFFECT OF CALCIUM SULFATE ON LEACHED PORTIONS OF ALKALI SOILS

A considerable quantity of the same soils was placed on Buchner funnels and leached with distilled water until practically free from chloride and sulfate. The soil was then spread out to dry, and after becoming dry, portions were treated with calcium sulfate as in the preceding experiments. The results are shown in table 9.

Comparing the untreated portions of these soils with the results shown in table 1, it will be seen that in addition to the  $\text{Cl}$  and  $\text{SO}_4$ , a considerable part of the  $\text{CO}_3$  and  $\text{HCO}_3$  was also removed by leaching. Treatment with calcium sulfate after leaching lowered the  $\text{CO}_3$  and  $\text{HCO}_3$  still further, but in contrast to the effects on the unleached soil

TABLE 9  
EFFECT OF CALCIUM SULFATE ON ALKALI SOILS AFTER LEACHING  
(Parts per million)

$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ added <sup>a</sup>	$\text{CO}_3$	$\text{HCO}_3$	$\text{SO}_4$	Ca	pH
Soil 905					
None	75	335	140	30	9.4
2150	30	290	1252	230	8.8
4300	15	259	2420	575	8.4
8600	15	213	4859	1390	8.4
17200	Trace	229	8816	2945	8.3
Soil 2736					
None	240	320	113	5	9.6
2150	45	167	1281	115	8.8
4300	15	167	2501	560	8.4
8600	Trace	152	4907	1525	8.3
17200	0	152	8320	2940	7.4
Soil 2753					
None	420	305	49	10	9.6
2150	60	259	1258	25	9.1
4300	15	213	2451	355	8.5
8600	12	198	4886	1310	8.5
17200	Trace	198	8531	2820	8.3
Soil 2754					
None	45	412	134	5	8.6
537	30	198	335	10	8.9
1075	12	167	649	105	8.3
2150	0	167	1219	305	7.2



(table 8) the application of the largest amounts of calcium sulfate precipitated the normal carbonate completely. It is noteworthy, however, that to produce this effect, relatively large amounts of calcium sulfate were required.

It should be pointed out that the results of these experiments are not in complete harmony with those obtained by the application of gypsum in plot experiments at Fresno, California. The analyses of several hundred soil samples, taken about two months after gypsum had been applied and after the plots had been heavily flooded, have in all cases failed to show any considerable amount of soluble calcium occurring simultaneously with soluble  $\text{CO}_3$ . With the use of calcium sulfate in the laboratory experiments, on the other hand, substantial amounts of both soluble calcium and  $\text{CO}_3$  were found in certain portions of each soil. In the laboratory experiments the products of the reaction remained in the soil, whereas in the plot experiments they were largely leached out. It is probable that the simultaneous occurrence of soluble calcium and  $\text{CO}_3$  noted in the laboratory experiments, was due to the solvent effect of the sodium sulfate formed by the direct action of the calcium sulfate applied.

#### EXPERIMENTS WITH FERROUS SULFATE

As a result of preliminary experiments, Lipman and Sharp<sup>10</sup> suggested that ferrous sulfate might prove to be useful in the treatment of black-alkali soils. Various amounts of this material have been added to unleached portions of these soils. The iron salt was mixed with the soil in the dry state, and then distilled water was added in the ratio of 1:5. After shaking for one hour the solutions were filtered and analyzed, with the results shown in table 10.

It will be noted that ferrous sulfate effectively reduced the alkalinity of each soil. Being a salt of a weak base and strong acid, ferrous sulfate gives an acid solution when dissolved in water. It is interesting to note that the effects produced by this material appear to be similar in nature to those produced by dilute solutions of  $\text{H}_2\text{SO}_4$  (compare tables 7 and 10). The concentration of soluble  $\text{CO}_3$  was reduced by the treatment, while that of  $\text{HCO}_3$  was substantially increased. With those soils relatively high in  $\text{CaCO}_3$  (905 and 2736) the addition of the largest amounts of ferrous sulfate produced the greatest increases in  $\text{HCO}_3$ , but such was not the case with the other soils.



TABLE 10  
EFFECT OF FERROUS SULFATE ON ALKALI SOILS  
(Parts per million)

Ferrous sulfate added	CO <sub>3</sub>	HCO <sub>3</sub>	Ca	pH
Soil 905				
None	210	610	5	9.4
1235	60	701	152	8.6
1875	15	945	200	8.6
3475	0	1113	330	8.1
6950	0	1494	690	8.0
Soil 909				
None	255	945	2	9.6
1735	45	1037	22	9.0
2605	Trace	1128	40	8.2
3475	0	1159	85	8.2
Soil 2736				
None	810	656	5	9.8+
1735	330	1067	12	9.5
3475	15	1540	20	8.6
6950	0	1860	385	8.0
Soil 2753				
None	1200	1281	7	9.8+
3475	285	2181	5	9.6
5210	0	2577	27	7.9
6950	0	2623	45	8.0
13900	0	976	295	7.0
Soil 2754				
None	420	778	7	9.8+
1735	105	1003	5	9.4
2605	15	1128	10	8.3
3475	0	1250	15	8.1
6950	0	1037	317	7.5

Since ferrous sulfate is a by-product in the manufacture of galvanized iron wire and similar materials and one for which present uses are limited, it is possible that this substance might prove to be useful in the practical treatment of black-alkali soils. It is important to state in this connection that an excess of soluble ferrous iron is considered to be extremely toxic to plants. It seems, however, from the limited study given this phase of the subject, that black-alkali soils have the power of precipitating very large amounts of iron, and it is probable that any excess which might be added could be leached into the subsoil and would tend to overcome the alkalinity of the subsoil. Since, as pointed out more fully below, flooding and drainage



is almost as essential to the reclamation of black-alkali soils as is the application of a neutralizing substance, the danger of using an excess of ferrous sulfate may not be great.

When a black-alkali soil is treated with ferrous sulfate a gelatinous precipitate is formed, which probably consists of a mixture of ferrous carbonate, ferrous hydrate and ferrous oxide. While each of these compounds is relatively insoluble in water, it is possible that their solubility in the soil solution might be considerable, especially if large amounts of  $\text{CO}_2$  be present. Under these conditions the concentration of ferrous ions might become sufficiently high to be toxic to plants. In order to gain some light on this point an excess of ferrous sulfate was added to portions of soils 2753 and 2754. After leaching out the excess of salts, it was found that barley seeds germinated readily in each soil, developed an extensive root system and continued to grow normally throughout the brief experimental period (two weeks); in fact, fully as good growth was secured as in portions of the same soils previously leached with a solution of calcium sulfate. Untreated portions of the soils, on the other hand, proved to be extremely toxic to barley, germination having failed in every trial. Moreover, it seems probable that under the conditions prevailing in natural alkali soils, any ferrous iron compounds would soon undergo oxidation with the formation of the less toxic ferric compounds. The iron would probably soon be precipitated as ferric hydrate and ferric oxide. It is planned to test this material more fully in plot experiments.

#### EXPERIMENTS WITH SOLUBLE ALUMINUM

It is well known that trivalent salts, such as those of aluminum, are powerful flocculents for soil colloids. Scofield<sup>17</sup> has suggested the use of aluminum salts as a treatment for certain badly deflocculated soils of the semi-arid region. Since soluble aluminum salts are precipitated as  $\text{Al}(\text{OH})_3$  by alkaline solutions, the alkalinity of the solution being itself reduced at the same time, it is possible that treatment with aluminum salts might prove effective on black-alkali soils. Ordinary potassium alum was used in these experiments. The dry salt was mixed with the soil and the mixture then extracted with water as in the previous experiments. The results are shown in table 11.



TABLE 11  
EFFECT OF ALUM ON\*ALKALI SOILS  
(Parts per million)

Alum added	CO <sub>3</sub>	HCO <sub>3</sub>	Ca	pH
Soil 905				
None	210	610	5	9.4
742	60	778	140	8.6
900	45	839	145	8.5
2966	0	1235	325	8.2
5932	0	1708	632	8.2
Soil 909				
None	255	945	2	9.6
742	105	1022	15	8.7
1483	15	1159	25	8.4
2966	0	1190	72	8.2
Soil 2736				
None	810	656	5	9.8+
1983	210	1464	30	9.5
2503	105	1616	25	9.0
5932	0	1815	297	8.2
Soil 2753				
None	1200	1281	7	9.8+
2966	315	2486	15	9.5
5932	0	2760	30	8.3
11864	0	1937	40	8.0
Soil 2754				
None	420	778	7	9.8+
742	225	914	10	9.4
1483	75	1159	10	8.8
2966	0	1281	20	8.3

It will be noted that the addition of alum reduced the alkalinity effectively. Since aqueous solutions of aluminum salts are acidic like those of iron salts, their chemical effect on black-alkali soils appears to be of a nature similar to that produced by dilute solutions of H<sub>2</sub>SO<sub>4</sub> and of ferrous sulfate. If the price is not prohibitive these experiments suggest that soluble aluminum salts may possibly find practical application as a treatment for black-alkali soils.

#### EXPERIMENTS WITH CO<sub>2</sub>

Lipman and Sharp<sup>9</sup> showed that the nitrogen-fixing organisms may be quite active in soils that contain comparatively high concentrations



of various sodium salts. Lipman and Gericke<sup>11</sup> found that the application of manure may stimulate the growth of crops on an unproductive black-alkali soil. It has frequently been observed that certain crops, alfalfa in particular, are capable of making reasonably good growth on soils which contain considerable amounts of sodium carbonate, provided the concentration is not too high during the early stages of growth. Since it is well known that the formation of  $\text{CO}_2$  by micro-organisms may be stimulated by the application of manure, and that  $\text{CO}_2$  is given off by the roots of growing plants, it is of interest to study the effects of  $\text{CO}_2$  on black-alkali soils.

Two-hundred-gram portions of the unleached soils were shaken for one hour with 1000 cc. of distilled water that had been partially saturated with  $\text{CO}_2$ . The amounts of  $\text{CO}_2$  contained in the water are recorded as parts per million of the dry soil (table 12).

TABLE 12  
EFFECT OF  $\text{CO}_2$  ON ALKALI SOILS  
(Parts per million)

$\text{CO}_2$ added	$\text{CO}_3$	$\text{HCO}_3$	Ca	pH
Soil 905				
None	210	610	5	9.4
2200	105	1921	125	9.6
4400	45	2486	200	9.0
8800	0	3690	405	8.2
Soil 909				
None	255	945	2	9.6
2200	105	1815	30	9.4
4400	0	2638	60	8.2
Soil 2736				
None	810	656	5	9.8+
2200	420	1998	37	9.6
4400	120	2669	75	9.4
8800	0	5154	130	8.1
Soil 2753				
None	1200	1281	7	9.8+
4400	570	3279	5	9.6
8800	0	5871	80	8.1
Soil 2754				
None	420	778	7	9.8+
2200	135	1693	10	9.5
4400	Trace	2623	20	8.4
8800	0	3538	65	8.0

It will be seen that as the amount of  $\text{CO}_2$  was increased the soluble  $\text{CO}_3$  gradually decreased and finally disappeared from each soil and at the same time the  $\text{HCO}_3$  markedly increased. The OH-ion concentration was reduced by  $\text{CO}_2$ , the extracts of each soil finally giving a pH-value of approximately 8.0. The solubility of calcium was also increased, the increases in the different soils being roughly proportional to their content of  $\text{CaCO}_3$ . It is especially noteworthy that considerable amounts of calcium were dissolved in soils 905 and 2736 without the pH-value of the solution falling below 9.0.

These results suggest, in harmony with Hibbard's data,<sup>6</sup> that the beneficial effect of manure on black-alkali soil may be due, in part at least, to the  $\text{CO}_2$  that is formed in its decomposition, and that the  $\text{CO}_2$  given off by the roots of growing plants may be of some importance in lowering the OH-ion concentration, particularly in that portion of the soil solution which is in contact with the roots.\*

Since a solution of  $\text{NaHCO}_3$  readily passes into  $\text{Na}_2\text{CO}_3$  upon evaporation, and since the transition is hastened by high temperatures such as frequently occur in alkali regions, the effect of manure is likely to be temporary. If the soil contains considerable amounts of  $\text{CaCO}_3$  and if it is leached after the manure has undergone decomposition, the bicarbonate may possibly be washed out with a resulting permanent benefit to the soil.

### GENERAL DISCUSSION

In the sulfonation experiments the decrease in soluble  $\text{CO}_3$  and  $\text{HCO}_3$  may be ascribed to the action of  $\text{H}_2\text{SO}_4$  formed by the oxidation of the sulfur. Calculating the  $\text{SO}_4$  equivalent of the losses in  $\text{CO}_3$  and  $\text{HCO}_3$  and comparing this quantity with the amount of  $\text{SO}_4$  actually formed, we find that in practically all cases the latter was much greater than the former. With the exception of the early period of the oxidation process, from 75 to 100 per cent more  $\text{SO}_4$  was formed than is required to effect the losses in  $\text{CO}_3$  and  $\text{HCO}_3$  noted. Similarly, the amounts of Ca precipitated by the soil where calcium sulfate was added, were usually at least twice as much as

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\* Pure water at equilibrium with  $\text{CaCO}_3$  with the latter present in excess, gives a reaction as alkaline as pH 8.6 to 8.8, but by merely passing  $\text{CO}_2$  through the suspension the pH-value of the solution can be easily reduced to a point well on the acid side of neutrality. It does not follow, therefore, that the soil solution around the roots of plants growing in calcareous soil in either humid or arid climates is necessarily alkaline.



can be accounted for by the decreases in soluble  $\text{CO}_3$  and  $\text{HCO}_3$ . Soil 905, in fact, appears to have the power of neutralizing  $\text{H}_2\text{SO}_4$  and precipitating Ca in amounts several times as great as would be expected by its soluble  $\text{CO}_3$  and  $\text{HCO}_3$ .

It is commonly held that  $\text{Na}_2\text{CO}_3$  forms adsorption compounds with soils. If such compounds occur in these soils, a part of their ability to neutralize acid and precipitate calcium could be accounted for on this basis. The data obtained by the use of  $\text{H}_2\text{SO}_4$  solutions indicate, however, that a considerable part of the acid was used up by substances other than carbonates at all stages of the reaction.

In a previous paper from this laboratory<sup>8</sup> it was suggested that complex silicates relatively high in sodium may occur in black-alkali soils. Such compounds, if present, would probably react readily with dilute acids. When treated with calcium sulfate the calcium of the added salt would tend to replace sodium, the former passing out of solution and the latter passing into solution. The preceding data strongly suggest that reactions involving double decomposition actually took place in each of these soils, that a part of the added acid was neutralized and a part of the calcium of the calcium sulfate was fixed by substances tentatively designated as alkaline silicates, and that these reactions took place even in the presence of soluble  $\text{CO}_3$ .

Whatever may have been the true nature of the reactions, computations show that the increases in  $\text{HCO}_3$  brought about by the various  $\text{H}_2\text{SO}_4$  solutions were sufficient to account for only a part of the acid used up. This seems difficult to explain if adsorbed carbonate was the only constituent other than soluble carbonate and  $\text{CaCO}_3$  involved in the reaction.

As a result of the several studies that have now been made on the various inter-relationships of salts and soils, it seems clear that black-alkali soils must be regarded as being sodium-saturated soils. Not only do they contain an excess of soluble sodium salts, but the finer fractions of the solid components are predominantly sodium compounds as well. These latter are extremely reactive with various chemical reagents and produce highly alkaline solutions when treated with relatively pure water. A more extended discussion of this phase of alkali soils will be given in a separate paper by Cummins and Kelley.<sup>4</sup>

Since the reactions which take place when black-alkali soils are treated with an acid or with calcium sulfate certainly involve both

the normal carbonate and the bicarbonate of sodium, and probably alkaline silicates and adsorbed carbonates as well, it is safe to conclude that much greater amounts of the neutralizing substance will ordinarily be required than a mere determination of the water-soluble  $\text{CO}_3$  would indicate.

These experiments show that the oxidation products of elemental sulfur and sulfuric-acid solutions lowered the concentration of sodium carbonate similarly in each of the five different alkali soils, but that after a part of the carbonate had been removed the sulfuric-acid solutions were the more effective of the two. With each soil except 905, the sulfur oxidation products finally removed the last trace of soluble  $\text{CO}_3$ . Since, however, these products attacked both normal carbonate and bicarbonate somewhat simultaneously, whereas sulfuric-acid solutions differentiated between these compounds, attacking the latter only after the former had been decomposed, a considerably greater amount of the sulfur oxidation products than of the sulfuric acid was required to remove the last trace of soluble  $\text{CO}_3$ .

While calcium sulfate lowered the concentration of soluble  $\text{CO}_3$  in each soil, the experimental results indicate that it is not possible to precipitate the carbonate completely with this material, so long as the concentration of neutral sodium salts remains excessive. Similar conclusions have recently been drawn by Hibbard.<sup>6</sup> Moreover, the results suggest that soils high in black alkali, although practically free from neutral salts, may also require leaching. Unless the soil be leached, the concentration of sodium sulfate, formed as a result of the treatment, may become so high as to hold a considerable part of the  $\text{CO}_3$  in solution.

As bearing on the practical application of these results, it is important to remember that crops produce a much deeper root system when grown in semi-arid than in humid regions. Not infrequently the roots of annuals penetrate to a depth of three or more feet and the roots of perennials, alfalfa and fruit trees for example, commonly grow to a depth of several feet. Moreover, the ability to develop a deep root system seems to be essential to the well-being of the plant. If the subsoil relatively near the surface is impermeable or uncongenial to root development, the growth of crops is likely to be seriously restricted. As is well known, black-alkali soils are usually underlaid by excessively alkaline subsoils, and a high degree of alkalinity may occur in successive layers of the subsoil to a depth of several feet. In



addition, one or more of the neutral salts are commonly present in black-alkali soils in toxic concentrations.

From the above discussion it seems evident that the reclamation of black-alkali soils is likely to involve at least two steps: (1) the application of a neutralizing substance in quantity sufficient to overcome the alkalinity of the soil and of the subsoil to the depth necessary for the normal development of the crops to be grown; (2) flooding and drainage. If elemental sulfur or sulfuric acid be applied to the surface of a soil in amounts sufficient to neutralize the injurious alkalinity present in both the soil and the subsoil, an acid condition of the surface soil must inevitably result unless other alkaline substances such as  $\text{CaCO}_3$  are present. On the other hand, an excess of gypsum may be applied to the surface and leached downward, thus ameliorating the subsoil without affecting the surface soil adversely.

Finally, as already pointed out, the silicates that naturally occur in black-alkali soils are probably saturated with sodium, the readily replacable calcium having already been substituted by sodium in nature. Treating such a soil with an acid, though it may effectively neutralize the alkali carbonates, cannot restore the calcium to the soil silicates, and there is much evidence that certain calcium silicates play an extremely important rôle in normal soil processes. There can be but little doubt that soil silicates saturated with calcium, or largely so, possess superior physical properties and promote conditions especially favorable for normal plant growth.

In view of these facts, we believe that gypsum, although considerably less effective chemically as a means of removing soluble carbonate, may in the long run prove to be preferable to elemental sulfur or sulfuric acid as a treatment for non-calcareous black-alkali soils. The fact that enormous deposits of gypsum occur in numerous places not far distant from the areas of black-alkali soils is also a factor to be considered. With alkali soils which contain an excess of  $\text{CaCO}_3$ , or in which the injurious alkalinity does not extend into the subsoil, it is possible that elemental sulfur or sulfuric acid may prove quite as effective as gypsum, and in some cases even more so. It is also possible that a treatment consisting of a combination of elemental sulfur and gypsum may prove superior to either alone.

It was also found that both ferrous sulfate and alum effectively neutralized the alkalinity of each soil, but the determination of the practical value of these materials necessitates further investigation.

## SUMMARY

1. Elemental sulfur undergoes reasonably active oxidation in alkali soils which contain relatively high concentrations of various sodium salts. It was found that the sulfur oxidation products finally neutralized the last trace of soluble  $\text{CO}_3$  in every soil studied except one.
2. With the use of sulfuric-acid solutions, normal carbonate was first converted into bicarbonate, whereas the sulfur oxidation products decomposed normal carbonate and bicarbonate simultaneously.
3. The addition of calcium sulfate produced a substantial lowering of the soluble  $\text{CO}_3$ , but it failed to reduce the alkalinity completely except when used in conjunction with leaching.
4. When considered on the basis of chemical equivalents, sulfuric-acid solutions were somewhat more effective than the sulfur oxidation products and considerably more so than calcium sulfate. The results indicate that each of these materials reacts in black-alkali soils with substances other than carbonates. The sulfur oxidation products and calcium sulfate also decompose bicarbonates, and each appears to react with alkaline silicates as well.
5. The amount of these materials that must be added is apparently considerably greater than a determination of the water-soluble alkalinity would indicate, and the excess appears to vary in different soils.
6. Since injurious alkalinity commonly occurs in the subsoil as well as in the surface soil, the successful treatment of black-alkali soils involves the necessity of considering the subsoil. Gypsum, although less effective chemically than elemental sulfur or sulfuric acid, may nevertheless be preferable in practice, since it is frequently necessary to employ leaching and the alkaline silicates present are capable of being converted into calcium silicates by the action of calcium salts. These latter silicates probably perform highly important functions in soils.
7. Both ferrous sulfate and alum neutralized the  $\text{CO}_3$  in each soil. The results indicate that either of these materials might be useful in the treatment of black-alkali soil. An excess of soluble ferrous iron or aluminum is, however, considered to be toxic to plants. Moreover, the precipitate formed by each of these materials



is gelatinous and may produce undesirable physical properties, especially where large amounts of black alkali occur. Culture experiments extending over a period of two weeks indicate, however, that the soil after treatment with ferrous sulfate and leaching, may be a favorable medium for growth. Further studies must be made before the practical value of these materials can be said to be established.

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TECHNICAL PAPER No. 3

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BY  
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These studies were first suggested some years ago by a field and laboratory comparison of the soils from the various plots of a fertilizer experiment at the Citrus Experiment Station, Riverside, California. The plot which has been treated with sodium nitrate annually for fifteen years has for several years possessed markedly different physical properties from those of adjacent plots. The soil, especially near the end of the irrigation runs, has become relatively impermeable, hard when dry and puddled when wet. It was suggested that the change in the soil consisted substantially in the displacement of some of the calcium in the soil silicates by the sodium of the  $\text{NaNO}_3$  applied, and that these new sodium silicate combinations were either colloidal in nature, or that conditions were suitable for their hydrolysis, whereby alkalinity has been produced with its attendant deflocculating effect upon the clay particles. Laboratory studies on the soil from this plot showed that it has indeed undergone marked physical modification. The rate of percolation of water downward, the rise of water by capillarity, etc., were much slower than with the soil from adjacent plots that have not been treated with sodium salts.

In attempting to prepare soil comparable with that of the  $\text{NaNO}_3$  plot, virgin soil taken nearby was first artificially leached in the laboratory with solutions containing neutral sodium salts and then with pure water to remove the excess of electrolyte. Almost immediately after

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\* Paper No. 100, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

the water was applied, the rates of percolation decreased, the soil masses swelled and dark-colored, alkaline percolates were obtained.\* Frequently these percolates became quite turbid with finely divided suspended matter, and after some days percolation usually ceased entirely. The soil residuum after such treatment presented a sterile, "baked" appearance upon drying, not unlike natural alkaline soils *in situ*.

Recently similar observations have been reported from other laboratories. It is interesting to note that very similar ideas have been developed by totally independent workers. The earlier literature contains many references to isolated aspects of the subject, but only a few of these need be reviewed here.

Van Bemmelen<sup>44</sup> noted a decrease in the rate of percolation when salts were washed from clay, hydrated oxides of tin, silica, etc., and that the dispersion of the colloids was very marked. He reported that the reaction was reversible; that is, the colloids could be flocculated again by treatment with salts. Mayer<sup>22</sup> had previously noted that treatment with either NaOH or NaCl and then with water, produced similar puddling effects in natural soil, whereas  $\text{Ca}(\text{OH})_2$  caused flocculation. Warington<sup>46</sup> called attention to the fact that leaching an acid-treated soil with water results in a similarly dispersed system. Hissink<sup>16</sup> reported that both KCl and NaCl decreased the permeability of the soil. Sharp<sup>47</sup> noted a very marked puddling effect produced by leaching salts from soils in tanks, and concluded that the physical effects produced were largely due to the markedly colloidal properties of the sodium compounds formed in the soil.

In a study previously reported on the chemical effect of salts on soils, data were presented confirming the well known replacement of bases between soils and neutral salt solutions.<sup>22</sup> A stoichiometric relationship was found to hold between the cation removed from a salt solution and the bases set free from the soil. This relationship can be expressed both graphically and mathematically, and when so expressed conforms with the established laws governing replacement and some metathetical reactions.

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\* The chemical nature of the dark colored extracts which are obtained from natural alkaline soils, and which are produced so abundantly in semi-arid soils by artificial means, has been insufficiently studied. The solutions are colloidal and contain much organic matter, but the relationship of the latter to the chemistry of alkali soils is not clear at present. This, and other aspects of the subject are being studied further at the Citrus Experiment Station.



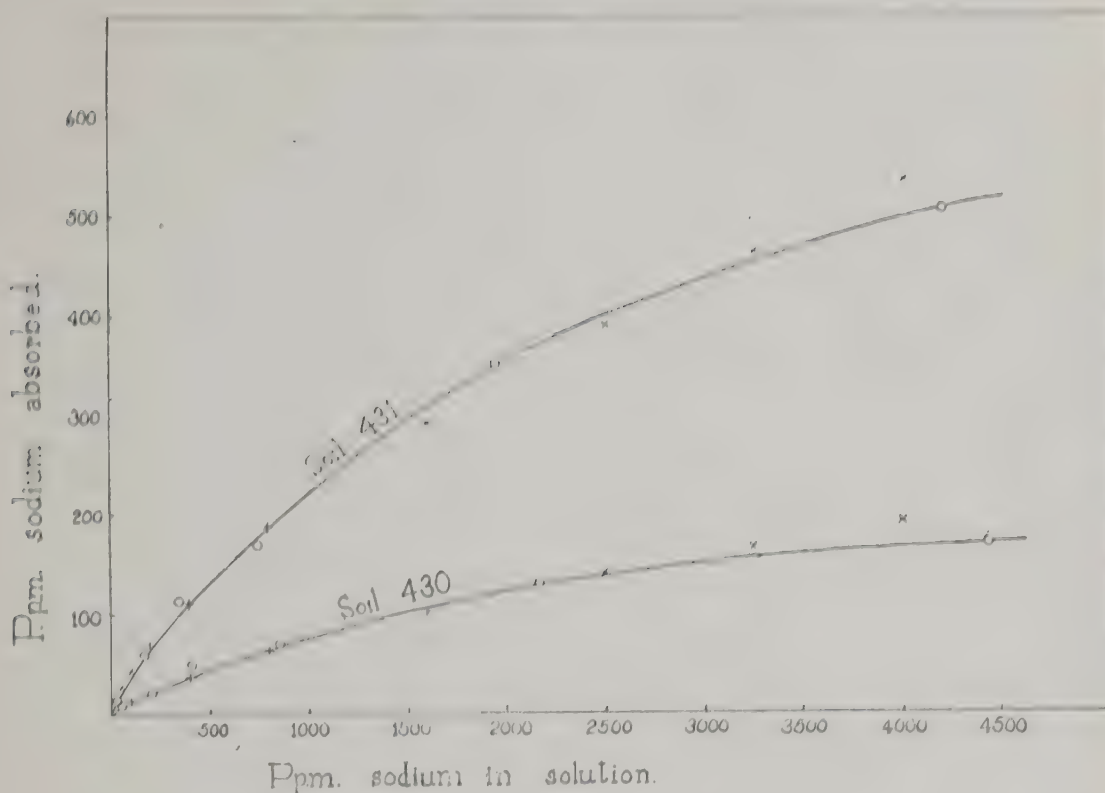


Fig. 1.

Curves showing the relation between sodium absorbed and sodium in solution at equilibrium. The circles represent the actual experimental data; the crosses represent the values calculated from the equations referred to in the text.

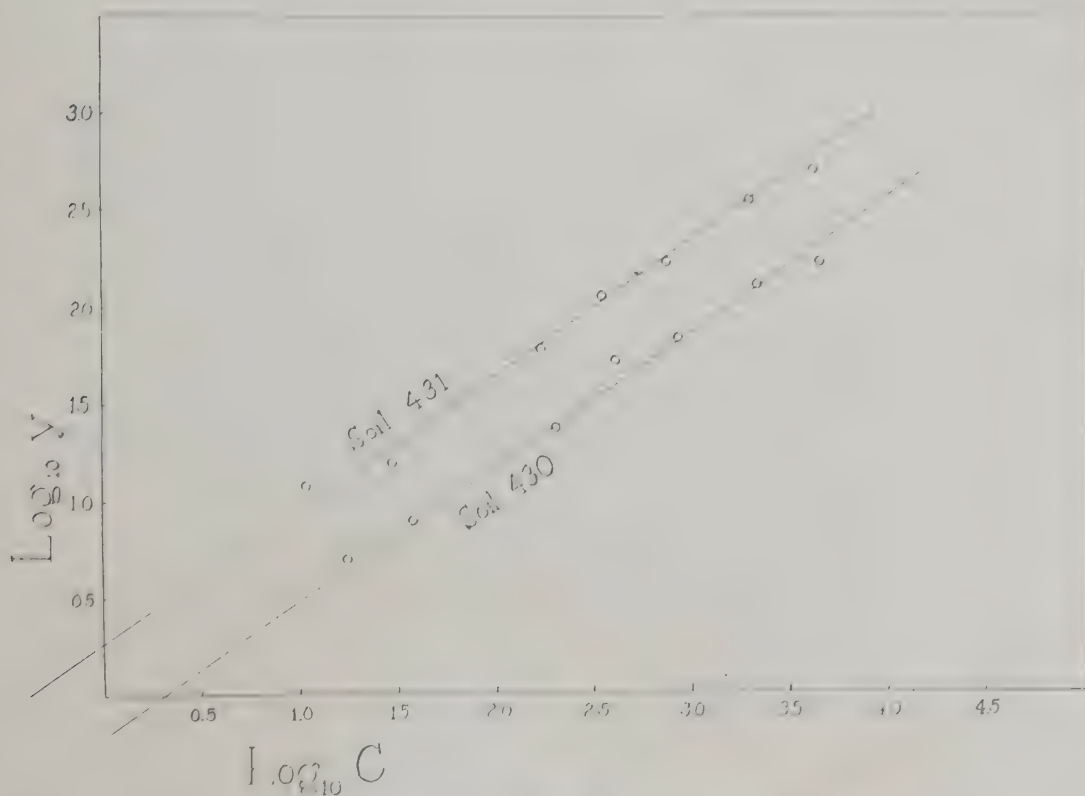


Fig. 2

Curves of figure 1 plotted logarithmically.

For example, when a soil was shaken to equilibrium with a solution of sodium chlorid, the sodium fixed was found to be an exponential function of the concentration of the sodium salt in the solution at equilibrium. As the total amount of calcium and the other bases brought into solution was found to be chemically equivalent to the sodium fixed, it is evident that the bases replaced are likewise a function of the concentration of the sodium salt. This confirms the work of Eichhorn,<sup>11</sup> Van Bemmelen,<sup>45</sup> Lemberg,<sup>21</sup> Wiegner,<sup>47</sup> Rice,<sup>36</sup> Sullivan,<sup>42</sup> and many others who have shown that basic exchange is a very general phenomenon in soils as well as in a wide variety of other substances.

The results obtained with two soils from southern California are shown graphically in figure 1. The exponential nature of the curves and their similarity are shown more clearly by plotting logarithmically, whereby two practically parallel straight lines are obtained.

The empirical equations expressing the relations between the sodium fixed and the concentration of the sodium chlorid in solution at equilibrium, are for the two soils respectively:  $\frac{y}{m} = 1.905 C^{0.68}$  and  $\frac{y}{m} = 0.631 C^{0.69}$ ; where  $y$  = the amount of sodium fixed,  $m$  = the weight of soil, and  $C$  = the concentration of sodium chlorid in solution at equilibrium.\* Freundlich<sup>12</sup> has emphasized the general applicability of this type of equation for a wide variety of adsorption phenomena and has styled the curves of such equations "adsorption isotherms," since they express the relationships of such reactions as the adsorption of iodine by starch, picric acid by silk, etc. Some soil investigators (<sup>15, 24, 34</sup>) have concluded, therefore, that basic exchanges between salt solutions and soils are essentially processes of physical adsorption. A review of numerous and varied chemical reactions, however, shows that many purely chemical reactions proceed in a similar manner.

In 1884 Ostwald<sup>33</sup> showed that the solubility of alkaline earth sulfates in acids is an exponential function of the concentration of the acid. Hall and Gimingham<sup>14</sup> showed the same relationship in the

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\* The equations were obtained by plotting the values of the log  $[\text{NaCl}]$  versus the log  $[\text{Na}]$  fixed. From the approximately straight lines thus obtained, the constants  $k$  and  $1/p$  in the general equation  $\frac{y}{m} = kC^{1/p}$  are estimated. The values of  $k$  are given by the intercept of the straight lines on the  $y$ -axis, and the value of the exponential constants  $1/p$  are numerically equal to the slopes of the curves.



replacement of bases by ammonia in soils. Prescott<sup>35</sup> also demonstrated a similar relationship in the reaction of oxalic acid with soils in the presence of dilute nitric acid. The general applicability of the exponential curve to biological phenomena has been shown by Moore and Bigland.<sup>30</sup> Most conclusive of all are Meyerhoffer's<sup>26, 27, 28</sup> extensive investigations on reciprocal salt pairs. He studied simple systems, such as  $K_2CO_3 + BaSO_4$ , and other metathetical equilibria, and found the reactions of many such purely chemical changes to follow exponential curves.

It is concluded, therefore, without discussing the nice differences between physical adsorption and chemical combination, that such typical basic exchanges as take place between a soil and a salt solution are primarily chemical in nature in the sense that the exchange of bases is stoichiometric in character and that the products of the reaction do not show marked differences from ordinary chemical compounds.

If we consider the reaction between a soil and a salt solution to be chemical, then the soil must undergo a change reciprocal in nature to that taking place in the solution. That is, as calcium replaces part of the sodium in the solution, sodium replaces part of the calcium in the soil. Essentially the process consists in the building up of a system of solid particles relatively richer in sodium and poorer in calcium (also Mg and K) than that originally present in the soil. Although the actual masses of the bases replaced are small in comparison with the total soil mass, the changes are sufficiently marked to produce profound physical difference in the soil. This difference is especially evident when the soil mass is freed from the excess of electrolyte. Whereas the calcium-silicate system is only slightly soluble and relatively stable, the sodium-silicate system is unstable, easily hydrolyzable and apparently capable of existing in the colloidal state.

In the paper cited<sup>19</sup> it was also noted that some soils may give distinctly alkaline extracts after treatment with neutral salts.\* The

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\* As is well known, many soils in humid regions impart an acid reaction to neutral salt solutions. Replacement of bases has been shown to be quite as common with these as with soils from arid regions; but, whereas calcium, magnesium, and potassium are the principal bases replaced from both alike, monovalent salts apparently also replace aluminum, iron, and manganese from certain acid soils of humid regions, but not from semi-arid soils. It has been claimed, although not definitely proven, that the acidity of saline extracts of the former is due largely to the aluminum, iron, etc., brought into solution by the neutral salt. The fact that salt solutions effect different soils differently in regard to iron, aluminum, etc., suggests a difference in the fundamental structure and chemical nature of the compounds present.

production of alkalinity was so pronounced when certain experimental details were observed, that further work has been done to determine the origin of this alkalinity. While the exact nature of the chemical reactions involved has not been thoroughly established and further study is being devoted to the pure chemistry of the phenomena, a preliminary discussion of the problem seems desirable. It appears that similar processes occurring in nature may be responsible for much of the carbonate alkalinity in arid lands. Furthermore, the theory as developed later offers a rational explanation for the conversion of some saline soils into alkaline soils.

Recently Scofield and Headley<sup>39</sup> published a paper on the physical condition of soils in relation to the alkali problem, considering especially the impermeability of soils to water produced in the course of reclamation by flooding and draining. These authors present data showing that neutral sodium salts produce three marked effects, namely: (1) the rate of percolation of water through soils is reduced by previous treatment with salts; (2) the percolate becomes turbid; (3) the percolate becomes strongly alkaline. The authors emphasized the deterioration produced in the physical properties of saline soils when the soluble salts are leached out by flooding or irrigation, but they neglected to discuss the possibility that such reactions are a potent agency in the generation of sodium carbonate. They confirm the facts that the alkalinity is developed before all the neutral sodium salts are leached from the soil and that the rate of percolation declines sharply upon leaching with water. They also emphasize the fact that the addition of calcium or aluminum salts is an effective means of ameliorating or preventing the action of sodium salts.

Later Scofield<sup>38</sup> emphasized the occurrence of colloidal sodium silicates as important components of the salt complex of arid soils and suggested the use of soluble aluminum compounds in correcting the physical properties of soils rich in such sodium compounds. He concluded that relatively large amounts of aluminum salts may be added without danger of rendering the soil toxic.

Earlier papers by Mondésir,<sup>29</sup> Gedroits,<sup>13</sup> and Dominici<sup>10</sup> came to our attention after this work was well advanced. These papers are generally unavailable to American workers and deserve more detailed review because of the experimental and theoretical contributions to



the phenomena in question. Bobko<sup>3</sup> has later confirmed some of Gedroits' conclusions.\*

Mondésir investigated certain calcareous soils in France where sodium chlorid was brought to the land by sea winds, and found that 80 per cent of the total chlorids of a water extract was present as calcium chlorid. He concluded that the sodium of the sodium chlorid had been fixed by the soil, calcium having been set free, and that the resulting calcium chlorid was removed by rains. The sodium absorption compounds were then capable of reacting with calcium bicarbonate, the calcium taking the place of the absorbed sodium. In this way sodium bicarbonate was generated. Mondésir produced experimentally 100 grams of trona ( $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ ) from one kilogram of another calcareous soil, by twenty repetitions of the treatment, first with a solution of NaCl, then with water, and finally with a solution of  $\text{CO}_2$ . He apparently did not consider the possibility of the formation of sodium carbonate in the absence of calcium carbonate, nor that the sodium absorption compounds may themselves hydrolyze, yielding alkaline solutions.

Gedroits observed that alkalinity is developed in saline soils upon leaching out the salts with relatively pure water, and considered that  $\text{CaCO}_3$  plays an important part in the process. The rôle ascribed to  $\text{CaCO}_3$  is, however, different from the metathetical reaction wherein  $2\text{NaCl} + \text{CaCO}_3 = \text{Na}_2\text{CO}_3 + \text{CaCl}_2$ .† He considers this reaction to be of limited importance as a source of  $\text{Na}_2\text{CO}_3$  in soils. Rather, he concludes, "NaCl,  $\text{Na}_2\text{SO}_4$  and  $\text{CaCO}_3$  are not to be considered as primary sources of soda (sodium carbonate) in the soil; but the rôle of these salts is an intermediate one. Sodium replaces other bases (Ca, Mg, K) from the humates and silicates of the soil, saturating the compounds to a greater or less degree with sodium, and it is these new sodium compounds that function as the direct source of  $\text{Na}_2\text{CO}_3$ , since they dissolve and decompose in the soil solution, giving rise to small amounts of alkalinity. Under suitable conditions, large amounts of alkalinity may be produced by reacting with  $\text{CaCO}_3$ ."

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\* A paper entitled "Base exchange and alkalinity in Egyptian soils," by J. A. Prescott, published in *Cairo Scientific Jour.*, vol. 10, nos. 106 and 107, pp. 58-64, 1922, was received after our manuscript had been written. This paper contains an interesting discussion of this problem and verifies the general conclusions drawn herein. It also refers to several important investigations by European and African workers which had previously escaped our attention.

† Breazeale<sup>4</sup> has more recently discussed this reaction at considerable length.

On the basis of this theory, Gedroits suggests that alkaline soils arise from saline soils. The neutral salts first saturate the soil to a greater or less degree with sodium, at which point the formation of significant amounts of sodium carbonate and the structure characteristic of alkaline soils are hindered by an excess of these salts. If, however, the concentration of these salts undergoes strong reduction, the saline soil then changes into an alkaline soil, the hydrolytic products combining with  $\text{CaCO}_3$  under suitable conditions to form large amounts of  $\text{Na}_2\text{CO}_3$ .

In the practical reclamation of saline and alkaline soils he concludes that artificial leaching alone can accomplish its purpose with neither class of soil. Saline soils would indeed deteriorate by it, since the tendency would be for their conversion into alkaline soils. Finally, he concludes that a combination of artificial leaching with the addition of gypsum should suffice, but that a considerable excess of gypsum above that recommended by Hilgard is necessary in the case of black-alkali soils; that is, an amount of calcium sufficient to replace the sodium in the absorption compounds as well as to neutralize any sodium carbonate that may be already present.\*

Dominicis, like Gedroits, obtained phenolphthalein alkalinity by washing the excess of salt from sodium-saturated soils and demonstrated that the amount of finely divided suspended matter was closely correlated with the amount of alkalinity. He furthermore stated that these reactions are responsible for the origin of sodium carbonate in nature.

Dominicis' theoretical explanation of the process is as follows: sodium replaces calcium, magnesium, etc., in certain silicate and humate combinations in the soil, forming sodium "absorbati" or "absorbates."† which are colloidal in nature; strong electrolytes (for

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\* Loughridge<sup>22</sup> in 1897, in enumerating some of the reasons why gypsum has failed in the reclamation of some "black-alkali" soils, concluded that twice the theoretical amount of gypsum should be applied. It is doubtful whether the reason for this was known at that time. Apparently Loughridge considered that there must be more sodium carbonate present than can be determined directly. As pointed out by Kelley and Brown,<sup>18</sup> it is not always possible to estimate the amount of sodium carbonate in a soil by ordinary solution methods of analysis. However, the theory of Gedroits and the experiments herein reported suggest the need for a still greater amount of gypsum. [See <sup>20</sup>.]

† The term "absorbate," suggested by Dr. H. S. Reed in translating the Italian word "absorbati," is retained in this paper as a convenient means of referring to the reactive Na-Ca-silicate complexes occurring in arid soils. It is realized that the expression has no definite significance as an acid radical, in the sense of nitrate, sulphate, etc., but is retained until the chemical nature of the compounds can be established.



example, sodium chlorid) keep the sodium "absorbates" coagulated as hydro-gels in which form hydrolysis does not take place. Therefore, no alkalinity can be developed until the concentration of electrolyte is materially reduced. In the absence of electrolytes the "gels" pass into "sols." The sodium then dissociates and forms sodium hydroxide, which is the primary source of the alkalinity. This sodium hydroxide may be converted into sodium carbonate by the action of calcium carbonate, but the presence of limestone is not essential, since the conversion into carbonate readily takes place in its absence. In nature, the equilibrium between dissociated sodium and the non-dissociated "absorbates" is continually displaced because of the transformation of the hydrate into the carbonate by  $\text{CO}_2$ . The passage of the hydro-gels into hydro-sols and the action of the negative charge of the hydroxyl ions in dispersing the colloid and maintaining the stability of the dispersion, explain the deflocculation effects accompanying the formation of alkalinity and the phenomena by which alkaline soils may either become impermeable to water or be reduced almost to sand through the removal of the clay by erosion.

The essential differences in the theories of Gedroits and Dominicis are thus slight. Gedroits apparently considers that the hydro-gels can, in themselves, hydrolyze, and that calcium carbonate is necessary for any appreciable formation of sodium carbonate. Dominicis believes that only the hydro-sols are directly concerned in the formation of alkalinity and that the presence of calcium carbonate is immaterial. It should be pointed out that Dominicis performed his experiments with a soil which originally contained considerable amounts of  $\text{CaCO}_3$  but he is of the opinion, although no definite proof is given, that the  $\text{CaCO}_3$  was completely dissolved and removed from the soil by the preliminary extraction with neutral salts. In the light of the results reported below, it seems possible, however, that  $\text{CaCO}_3$  may have been responsible either directly or indirectly for a considerable part of the alkalinity which Dominicis noted.

#### EXPERIMENTAL RESULTS

##### *The rôle of $\text{CaCO}_3$ in the hydrolysis of sodium "absorbates" in the soil*

One-hundred-gram portions of two different soils, each of which contained no  $\text{CaCO}_3$ , were shaken (with amendments as noted) in

flasks with liter portions of N/5 NaCl solutions. The suspensions were allowed to settle out and the supernatant liquids siphoned off. These treatments were repeated five times, when pure water was substituted for the salt solutions and similar treatments continued. Tables 1 and 2 show the amounts of phenolphthalein alkalinity found in the supernatant liquids.

TABLE 1

ALKALINITY DEVELOPED BY EXTRACTING SOIL 430 WITH N/5 NaCl,  
THEN WITH H<sub>2</sub>O

Preliminary treatment	Secondary treatment	Order of secondary treatments cc. N/50 acid per liter (phenolphthalein)					
		1st	2nd	3rd	4th	5th	6th
Soil saturated with N/5 NaCl.....	H <sub>2</sub> O	Trace	0	0	0	0	0
Soil + CaCO <sub>3</sub> saturated with N/5 NaCl.....	H <sub>2</sub> O	3.7	19.0	24.5	20.0	10.3	3.1
Soil saturated with N/5 NaCl.....	CaCO <sub>3</sub> + H <sub>2</sub> O	1.0	12.0	16.4	12.0	5.5	3.2
Soil saturated with N/5 NaCl.....	CaCO <sub>3</sub> + N/5 NaCl	0	0	4.5*	26.0	24.5	16.0
Soil leached with H <sub>2</sub> O.....	H <sub>2</sub> O	0	0	0	0	0	0

\* After the second treatment H<sub>2</sub>O was substituted for N/5 NaCl.

TABLE 2

ALKALINITY DEVELOPED BY EXTRACTING SOIL 431 WITH N/5 NaCl,  
THEN WITH H<sub>2</sub>O

Preliminary treatment	Secondary treatment	Order of secondary treatments cc. N/50 acid per liter (phenolphthalein)						
		1st	2nd	3rd	4th	5th	6th	7th
Soil saturated with N/5 NaCl.....	H <sub>2</sub> O	0	0	0	0	0	0	0
Soil + CaCO <sub>3</sub> saturated with N/5 NaCl.....	H <sub>2</sub> O	0	0	9.0	22.6	27.0	22.2	19.0
Soil saturated with N/5 NaCl.....	CaCO <sub>3</sub> + H <sub>2</sub> O	0	0	9.3	17.3	25.0	20.3	17.4
Soil saturated with N/5 NaCl.....	CaCO <sub>3</sub> + N/5 NaCl	0	0	0*	0	11.7	27.0	28.5
Soil leached with H <sub>2</sub> O.....	H <sub>2</sub> O	0	0	0	0	0	0	0

\* After the second treatment H<sub>2</sub>O was substituted for N/5 NaCl.

Similar studies were made with Na<sub>2</sub>SO<sub>4</sub> and NaNO<sub>3</sub>. The results were similar to those obtained with NaCl, as is shown in tables 3 and 4.



TABLE 3  
ALKALINITY DEVELOPED BY EXTRACTING SOIL 430\* WITH N/5 Na<sub>2</sub>SO<sub>4</sub>,  
THEN WITH H<sub>2</sub>O

Preliminary treatment	Secondary treatment	Order of secondary treatments cc. N/50 HCl per liter (phenolphthalein)						
		1st	2nd	3rd	4th	5th	6th	7th
Soil saturated with N/5 Na <sub>2</sub> SO <sub>4</sub> .....	H <sub>2</sub> O	0	0	0	0	0	0	0
Soil + CaCO <sub>3</sub> saturated with N/5 Na <sub>2</sub> SO <sub>4</sub> .....	H <sub>2</sub> O	0	11.0	17.0	10.0	13.0	13.0	9.0
Soil saturated with N/5 Na <sub>2</sub> SO <sub>4</sub> .....	H <sub>2</sub> O	0	6.0†	11.5	5.0	9.0	9.0	5.0

† CaCO<sub>3</sub> mixed with the soil after first treatment with H<sub>2</sub>O.

TABLE 4  
ALKALINITY DEVELOPED BY EXTRACTING SOIL 430\* WITH N/5 NaNO<sub>3</sub>,  
THEN WITH H<sub>2</sub>O

Preliminary treatment	Secondary treatment	Order of secondary treatments cc. N/50 HCl per liter (phenolphthalein)						
		1st	2nd	3rd	4th	5th	6th	7th
Soil saturated with N/5 NaNO <sub>3</sub> .....	H <sub>2</sub> O	0	0	0	0	0	0	0
Soil + CaCO <sub>3</sub> saturated with N/5 NaNO <sub>3</sub> .....	H <sub>2</sub> O	0	15.0	27.0	21.5	20.0	18.0	13.3
Soil saturated with N/5 NaNO <sub>3</sub> .....	H <sub>2</sub> O	0	5.0†	17.0	12.0	12.0	11.2	7.3

\* Similar results were obtained with soil 431 except that a greater number of extractions with water was necessary before phenolphthalein alkalinity developed. The amount of alkalinity produced, however, was greater than with soil 430.

† CaCO<sub>3</sub> mixed with the soil after first treatment with H<sub>2</sub>O.

Appreciable amounts of phenolphthalein alkalinity were produced with both soils, only, however, when CaCO<sub>3</sub> was present. No very marked differences were obtained when the limestone was added to the soil either before or after the treatment with the neutral sodium salt.

Apparently, these data confirm Gedroits' conclusion that CaCO<sub>3</sub> is essential for the formation of an appreciable amount of Na<sub>2</sub>CO<sub>3</sub>. It was believed, however, that the experimental technique employed involved conditions so unlike those obtaining in nature, that conclusions drawn from such a study should not be considered final. Some means whereby the water is allowed to percolate through a soil

column was thought to approximate more nearly the natural leaching of a soil. Where this method was employed much more striking and totally different results were obtained.

*The Genesis of Sodium Carbonate*

In this study 5-kilogram portions of soil No. 430, taken from the Citrus Experiment Station site, and which contained no  $\text{CaCO}_3$ , were placed in large bottles and treated three times with 12½ liter portions of normal sodium chlorid solution, the supernatant liquid being siphoned off each time. The soil was finally drained on a large Büchner funnel, air dried, and pulverized. It was then placed in 3-inch tubes, without packing, and a constant head of pure water maintained on each soil column. On February 10, 1921, twenty-five hundred grams of air-dried soil was placed in tube 1, and an equal amount of the same soil was placed in tube 2 after adding 2 per cent of precipitated C. P. calcium carbonate and intimately mixing. The water percolated through the soil columns exceedingly slowly, but the volume of percolate from tube 2 was considerably greater than that from tube 1. The first portions of the percolates from both tubes were high in sodium chlorid. The percolate from tube 1 was not alkaline to phenolphthalein at first, while that from tube 2 was distinctly so. This was, however, evidently due to the reaction between calcium carbonate and sodium chlorid and should not be confused with the much greater alkalinity that developed later. In three weeks the percolate from tube 1 manifested alkalinity to phenolphthalein. the amount of which increased rapidly.

TABLE 5  
ALKALINITY DEVELOPED BY EXTRACTING SOIL 430 WITH NORMAL NaCl AND THEN PERCOLATING WATER THROUGH IT

Date	cc. of percolate obtained	cc. N/10 acid per liter (Phenolph.)	cc. N/10 acid per liter (Methyl orange)	Gm. $\text{Na}_2\text{CO}_3$ per liter	Gm. $\text{Na}_2\text{CO}_3$ total percolate
Soil alone—					
March 12.....	55	2480	5800	26.288	1.446
March 16.....	40	3450	7360	36.57	1.463
Soil + $\text{CaCO}_3$ —					
March 12.....	275	370	1000	3.922	1.078
March 16.....	800	100	500	1.06	0.848



Table 5 shows the extraordinarily high concentrations of sodium carbonate obtained on March 12 and 16. The soil without calcium carbonate yielded a percolate containing phenolphthalein alkalinity equivalent to 3.66 per cent sodium carbonate, while with calcium carbonate the highest concentration was 0.39 per cent sodium carbonate. When the total volumes of the percolates are considered, however, the tubes are seen to have yielded more nearly the same total amounts of sodium carbonate.

The results are considered to indicate: (1) that calcium carbonate is not essential to the formation of sodium carbonate in leaching a salt-saturated soil with water; (2) that important amounts of sodium carbonate may be formed in nature by the percolation of relatively pure water through soils that have been previously saturated with neutral sodium salts.

The production of alkalinity in this manner has not been generally recognized by soil investigators, but these results indicate that this is an important agency in "black-alkali" formation. The conditions necessary for the progress of the reaction are more simple and easily realizable in the state of nature than some of the many possible reactions that have been suggested by previous workers.<sup>1, 5, 6, 7, 31, 37, 41, 43</sup>

Percolation studies using soil saturated with NaCl were repeated several times with similar results. The general course of the percolations was always an initial rapid penetration of the water, with a lowering of the rate usually occurring long before any percolate had passed entirely through the soil column. When the soil was free from  $\text{CaCO}_3$  at the outset, the first portions of the percolates obtained were very concentrated with respect to NaCl and were not alkaline to phenolphthalein. High alkalinity in the percolates usually appeared some days after the first water passed through, although several weeks were frequently required, so greatly was the soil deflocculated. The production of alkalinity usually continued for several days or weeks, and then, if water continued to pass through at all, it began to diminish until in some cases no further phenolphthalein alkalinity was produced.

The rate of percolation was always very slow. With a heavier soil, No. 431, from La Habra, California, water sometimes failed to percolate through the column for a period of several weeks. Only 4 cc. of percolate were obtained from an almost neutral silt loam soil

from Indiana, after it had first been leached with NaCl solution. This small amount of liquid, however, was alkaline to phenolphthalein. An acid Indiana soil, giving a strong test for acidity by Comber's test<sup>8</sup> was first leached with NaCl, then dried and placed in a percolation tube. This soil gave a water extract with pH 5.0 and in the treatment with salt yielded copious amounts of aluminum and manganese in addition to calcium, magnesium, and potassium. Water percolated slowly through this soil and the percolate showed a progressive rise in pH value from 5 to 7.8. Thus, while this distinctly acid soil was not converted into a "black-alkali" soil, it became distinctly alkaline by treatment merely with NaCl and water. It also showed the same tendency to become deflocculated and impermeable to water as that shown by semi-arid soils.

Several attempts were made to increase the permeability of these soils by admixing them with three to four times their weight of sand, broken pottery, and other inert substances, but the dispersion of the colloidal material was so great that little better results were obtained by this means. In certain cases the deflocculated soil was removed from the tubes, air dried, pulverized, and returned to the tubes. Percolation after such treatment was scarcely improved. The physical properties of the soil apparently had been permanently altered by the changes that took place in the initial treatment with sodium chlorid.

In the preceding experiment no provision was made to exclude carbon dioxide and, because of the long time necessary to obtain measurable amounts of percolates, the alkalinity obtained was largely carbonate and bicarbonate alkalinity. To determine whether sodium hydroxide is the alkaline compound resulting directly from the hydrolysis of the sodium "absorbates" another percolation study was made.

Five kilograms of soil 430 was leached with many liters of normal sodium sulfate solution on a Büchner funnel. The soil was finally drained on the funnel by suction, air dried, and pulverized. It was then placed in a tube arranged for percolation in the absence of carbon dioxide and under reduced pressure.

To remove absorbed CO<sub>2</sub> from the dried soil, a stream of air, freed from CO<sub>2</sub> by bubbling through a concentrated solution of sodium hydroxide, was drawn through the soil for eighteen hours. Connection was then made with a bottle of carbon-dioxide-free water, without



breaking the partial vacuum in the apparatus. Water was immediately drawn over on the soil column and allowed to percolate downward by gentle suction. The first drops of percolate were obtained within a few hours and for some time the rate of percolation continued at the rate of about 10 cc. per hour. In two days faint phenolphthalein alkalinity appeared, the amount increasing rapidly, and the percolate became deeply colored. Ultimately it became impossible to draw any further liquid through the soil column.

The final percolate was strongly alkaline, giving a pH-value of 12.85, corresponding to a hydroxyl-ion concentration of  $7.13 \times 10^{-2}$  or a hydrogen-ion concentration of  $0.142 \times 10^{-12}$ . This solution gave a titration figure corresponding to 10,500 cc. of N/10 acid to neutralize 1 liter to phenolphthalein, and 4100 cc. additional acid to neutralize 1 liter to methyl orange. The results, therefore, correspond to the titration of a mixture of hydrate and carbonate or silicate. The data are shown in table 6.

TABLE 6

PERCOLATES OBTAINED FROM SOIL 430 IN THE ABSENCE OF FREE CARBON DIOXIDE

Date	Character of percolate	Volume of percolate (cc.)	Test for SO <sub>4</sub>	Alkalinity cc. N/10 acid per liter	
				Phenolphthalein	Methyl orange
March 15	Muddy.....	35	Very high	0	45.0
March 15	Clear, straw color.....	10	Very high	0	50.0
March 15	Clear, straw color.....	20	Very high	0	.....
March 16	Clear, straw color.....	15	Very high	0	.....
March 16	Clear, light straw color..	10	Very high	Trace	.....
March 16	Clear, light straw color..	12	Very high	Trace	40.0
March 17	Clear, light straw color..	8	Very high	Trace	40.0
March 17	Muddy, dark color.....	25	.....	.....	.....
March 18	Dark color.....	2	High	350	900.*
March 18	Dark color.....	10	High	2060	3140.†
March 18	Dark color.....	5	High	600	.....
March 19	Very dark, free from suspended matter.....	20	High	10500	14600.‡

\* Carbonate and bicarbonate present.

† Largely hydrate or silicate.

‡ Hydrate and carbonate or silicate.

Additional confirmation of the presence of hydrate is indicated by the titrations made upon the dialysates of the final percolate obtained above, as given in table 7. The results of this experiment, therefore,

substantiate the “absorbate” theory, in that sodium hydroxide appears to be the primary source of the alkalinity developed on leaching a sodium-salt-treated soil with pure water.

TABLE 7  
ALKALINITY OF 10 CC. OF PERCOLATE AFTER DIALYSIS  
cc. N/10 acid

	Phenolphthalein	Methyl orange
1st dialysate.....	4.10	5.20
2nd dialysate.....	2.10	3.25
3rd dialysate*.....	Trace	.....

\* The liquid remaining in the dialyzing sack was not alkaline to phenolphthalein, and contained practically no sulphates.

The possibility that the alkalinity may have been produced by sodium silicate has not been excluded. Some of the alkaline extracts were both titrated and analyzed for carbon dioxide and silica, but the amounts available for these purposes were so small that accurate analyses were difficult. In the portions tested no appreciable amounts of silica were found. The percolates obtained from open tubes always contained actual carbonate, but generally in amounts somewhat smaller than that represented by the titration figures. Although sodium silicate may have been one of the initial decomposition products, this probably hydrolyzed immediately, NaOH and silicic acid being formed and the latter either remaining in colloidal solution or being precipitated. In either case the silica would be removed from active chemical action, and NaOH may, therefore, be considered the initial alkaline hydrolytic product of the “absorbate.”\* It is the removal of the hydroxyl-ions by the CO<sub>2</sub> of the air that insures the continued decomposition of the “absorbate” and accounts for the progressive accumulation of Na<sub>2</sub>CO<sub>3</sub>.

*Development of alkalinity from granitic rocks*

The exchange of bases between salt solutions and the unaltered minerals in rocks has been abundantly confirmed.<sup>9, 11, 21, 25, 32, 42</sup> It is interesting, therefore, to determine whether such a material as

\* Kahlenberg and Lincoln<sup>17</sup> have shown by freezing-point determinations and conductivity measurements that in solutions of the simple alkali silicates, hydrolysis into the hydroxide and colloidal silicic acid is practically complete in concentrations up to about 1/100 normal.



powdered granite will develop alkalinity upon treatment with sodium chlorid and subsequent leaching with water. It was believed that positive data on this point would furnish additional evidence in support of the "absorbate" theory.

For this experiment specimens of unaltered and of weathered granites were secured from Mt. Rubidoux, Riverside. They were ground to pass through a 60-mesh sieve, leached on Büchner funnels with normal sodium chlorid solutions,\* then dried and placed in tubes, as previously described for the soils. Alkaline percolates were obtained as shown in table 8. As a check on the solubility of the granites, pure water and normal sodium chlorid solutions were percolated through columns of untreated granite, but no phenolphthalein alkalinity was produced.†

TABLE 8

ALKALINITY OF PERCOLATES OBTAINED FROM POWDERED GRANITE PREVIOUSLY  
TREATED WITH NaCl

## Unaltered Granite

	Vol. of percolate (c.c.)	cc. N/10 acid per liter		Gm. Na <sub>2</sub> CO <sub>3</sub> per liter	Gm. Na <sub>2</sub> CO <sub>3</sub> in total volume obtained
		Phenolph- thalein	Methyl orange		
March 30.....	600	11.5	82.5	0.122	.073
March 31.....	800	12.0	84.0	0.127	.102
April 4.....	800	Trace	65.3	Trace	Trace

## Weathered Granite

March 30.....	2000	5.0	52.0	.053	.106
March 31.....	2500	4.5	23.5	.048	.120
April 4.....	2200	2.2	22.0	.023	.051

The results show that appreciable amounts of sodium carbonate may be formed by merely treating powdered granite with NaCl and then percolating water through a column of the material. This occurred both with fresh rock and with somewhat altered rock. The amounts of alkalinity produced were much smaller than with the soil

\* Notable amounts of calcium were found in the sodium chlorid percolates, indicating basic exchange.

† The fact that no phenolphthalein alkalinity was observed in the water percolates of the untreated granites should not be interpreted as meaning that the minerals in the rock are incapable of hydrolysis in pure water, but rather that the conditions of the experiment did not allow this phenomenon to be demonstrated. Portions of the finely ground rock shaken with carbon-dioxide-free water, gave alkaline reactions to phenolphthalein, especially if allowed to evaporate at ordinary temperature exposed to the atmosphere.

derived from these rocks, and the puddling effect, which was a notable feature of the soil, was not so apparent with the ground rock. This might be expected, however, since the replacement of bases in granite by NaCl was not as great as with the soil.

The behavior of these granites indicates that purely inorganic silicates, and probably not organic compounds, are largely concerned in the phenomena observed in the soils, and further, that the silicates involved need not necessarily be greatly altered or secondary minerals.\*

### *Development of alkalinity with pure minerals*

The results obtained with the powdered granites suggested a similar study with pure minerals. The work of the several investigators cited has established the fact that replacement reactions between quite stable and only slightly soluble minerals and saline solutions take place, although the extent of this metathesis is generally not great. With the soil studied and with the Rubidoux granites, calcium was the base most abundantly replaced by sodium. Therefore, several types of naturally occurring calcium silicates and calcium aluminum silicates were studied with respect to their behavior with solutions of NaCl. It was believed that in this manner some insight might be gained as to the nature of the compounds that may be involved in similar processes in soils.

Pure, crystalline specimens of the minerals were crushed and pulverized in an agate mortar. The grinding was continued until all of the material passed through a 100-mesh sieve. Solubility studies were made with these materials, employing carbon-dioxide-free water and N/10 NaCl solutions made up from the very carefully purified salt. Two-gram portions of the minerals were shaken with two liters of the solvent for four hours, the suspensions allowed to stand over night, reshaken on the following day for four hours, then filtered and analyzed. The reactions of the resulting solutions and the soluble calcium and silica were determined. The results are shown in table 9.

\* The chemical analyses of the two granites are:

	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	FeO	TiO <sub>2</sub>	MnO	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	Total
Fresh .....	74.69	12.42	0.33	2.25	.18	.12	1.18	.62	4.28	4.08	.04	100.19
Weathered	74.91	12.45	0.10	1.63	.20	.08	1.27	.44	4.29	4.07	.03	100.17

According to W. Harold Tomlinson, the petrographical composition of the fresh rock, which is classified as a quartz-monzonite, is in descending order of abundance: orthoclase, quartz, oligoclase, biotite, hornblende. No secondary minerals were detected by microscopical examination of the thin rock sections.



TABLE 9  
SOLUBILITY OF MINERALS IN WATER AND N/10 NaCl SOLUTION

Mineral	Solubility in water			Solubility in N/10 NaCl		
	Reaction to phenolphthalein	p.p.m. Ca	p.p.m. SiO <sub>2</sub>	Reaction to phenolphthalein	p.p.m. Ca	p.p.m. SiO <sub>2</sub>
Pectolite.....	Distinct rose....	3.4	3.7	Deep rose*.....	4.6*	5.0*
Apophyllite.....	Very deep rose	3.6	3.7	Very deep rose	8.8	9.0
Wollastonite.....	Pink.....	2.8	3.4	Distinct pink..	6.8	7.0
Natrolite.....	Distinct pink..	1.2	0.7	Distinct pink..	6.1	1.3
Thomsonite.....	Faint pink.....	1.5	0.6	Faint pink.....	11.1	1.4
Chabazite.....	Colorless.....	0.7	0.7	Colorless.....	34.0	1.1
Labradorite.....	Colorless.....	0.5	2.7	Colorless.....	4.0	2.4
Anorthite.....	Decided pink..	6.0	1.4	Rose.....	15.7	2.5

\* N/100 NaCl solution used.

Pectolite, apophyllite, and wollastonite are similar in being relatively soluble and easily hydrolyzable in pure water. The solubility of both calcium and silica was distinctly higher than was the case with the other minerals with the exception of anorthite. The concentration of hydroxyl-ions and the total titratable alkalinity were likewise greater. With the use of NaCl, however, the calcium replaced was not great with pectolite, apophyllite, or wollastonite, and an attendant increase in soluble silica was noted in each case.

On the other hand, chabazite and thomsonite, both of very low solubility in pure water, showed pronounced replacement of calcium by NaCl. At the same time, there was no marked increase in the solubility of the silica. With chabazite, 0.069 grams of calcium were found in the two liters of NaCl solution. Since the original two grams of the mineral contained but 0.158 grams of calcium, 43.6 per cent\* of the calcium had been replaced by sodium. It is not believed that such mild treatment as several hours digestion in a dilute saline solution at room temperature could have altered the fundamental structure of the silicate molecule, but rather that the resulting compound was similar structurally to the original calcium aluminum silicate, a portion of the calcium being merely substituted by sodium. Chabazite was the most reactive mineral studied, but thomsonite and anorthite showed considerable reactivity with NaCl.

\* Assuming a composition expressed by the formula  $\text{CaAl}_2\text{Si}_4\text{O}_{12}\cdot 6\text{H}_2\text{O}$ , which was approximately the case, since the mineral actually contained 19.47 per cent combined water as contrasted with the 21.32 per cent required by the formula.

The behavior of these minerals with sodium chlorid is strikingly similar to that of soils 430 and 431. Tests were, therefore, made by first leaching columns of the powdered minerals with solutions of sodium chlorid and then replacing the saline solutions with pure water. The phenomena observed were in some cases strikingly similar to those occurring in soil and corroborate the main assumptions of the “absorbate” theory.

The amount of material available for the percolation studies was limited and variable. In some cases only a few grams of material were used. The technique consisted in the use of small glass tubes in which the mineral powders were supported on a layer of pure quartz sand. Normal solutions of sodium chlorid were first percolated through the columns until the percolates no longer gave definite tests for calcium. Pure water was then poured into the tubes.

In the experiment with thomsonite, fifty grams of material was used. The NaCl solution percolated through this column was not alkaline to phenolphthalein, but immediately upon replacing the saline solution with pure water, a striking alkalinity was apparent in the percolate, quickly followed by a pronounced opalescence and then turbidity. This latter eventually became a very fine suspension, some of which settled out and proved to be pure silica. At the same time the rate of percolation decreased, as with soil, although the deflocculation was not nearly so great. Portions of the percolate were tested, with results as shown in table 10.

TABLE 10  
ALKALINITY PRODUCED BY LEACHING NaCl-TREATED THOMSONITE  
WITH PURE WATER

Percolate	Vol. cc.	cc. N/10 acid required to neutral- ize 1 liter		Phenolphthalein titration calculated as Na <sub>2</sub> CO <sub>3</sub> per liter	Actual Na <sub>2</sub> CO <sub>3</sub> in percolate
		Phenolph.	Methyl orange		
1st.....	45	60	112	0.635 gm.	0.023 gm.
2nd.....	150	40	80	0.424 gm.	0.063 gm.
3rd.....	35	20	56	0.212 gm.	0.007 gm.
4th.....	425	10	34	0.106 gm.	0.044 gm.
Total.....	655	.....	.....	.....	0.137 gm.

Similar results were obtained with chabazite. Only 1.5 grams of this mineral was available for the percolation test, yet this small amount yielded an abundant amount of calcium with the NaCl solution



and gave a pronounced alkalinity upon subsequent leaching with water. Anorthite behaved similarly, while the sample of labradorite tested, although larger in amount, did not yield nearly as much phenolphthalein alkalinity. Wollastonite, pectolite, and apophyllite gave alkaline percolates with sodium chlorid, and the amount of calcium taken into solution was noticeably smaller than with the other minerals. Upon leaching with water the alkalinity of the percolates increased somewhat, but not to the same extent as with thomsonite, chabazite, anorthite, and labradorite. The results are shown in table 11.

TABLE 11  
ALKALINITY PRODUCED BY VARIOUS MINERALS ON LEACHING WITH  
NaCl SOLUTIONS AND H<sub>2</sub>O\*

Mineral	Treatment	
	NaCl cc. N/10 acid per lit	H <sub>2</sub> O after NaCl er (phenolphthalein)
Pectolite.....	5.0	8.0
Apophyllite.....	10.0	15.0
Chabazite.....	none	20.0
Wollastonite.....	32.0	53.0
Anorthite.....	none	150.0
Labradorite.....	none	75.0

\* Unequal amounts of the different minerals were used. Therefore the amounts of alkalinity developed should not be regarded as being quantitatively comparable. Labradorite was used in the largest amount, but the quantity of anorthite was much in excess of that of the other minerals except labradorite. The quantity of chabazite used (1.5 gm.) was much less than that of any other mineral.

The results indicate that the calcium of the hydrated calcium aluminum silicates of the thomsonite-chabazite type is the most readily replaced by sodium, and that the resulting silicates, relatively richer in sodium, hydrolyze in pure water, acting then as a primary source of alkalinity. The two plagioclase feldspars studied, anorthite and labradorite, exhibited similar properties and acted in a strictly analogous manner, although the amounts of alkalinity yielded by these minerals, considering the quantities used, were smaller than with the zeolites.

Wollastonite, pectolite, and apophyllite, representing an anhydrous meta-silicate, an anhydrous zeolite-like mineral, and a hydrated alkaline zeolite, respectively, did not react freely with NaCl and,

consequently, having been relatively unsaturated with sodium, did not manifest greatly increased hydrolytic capacity with pure water. The distinct solubility of unaltered pectolite, apophyllite, anorthite, and wollastonite, in pure water, is, however, significantly greater than that of the other minerals tested, and is sufficient to make such minerals potent sources of alkalinity without the intermediate reaction with solutions of sodium salts. It is proposed to continue the study of pure minerals in an effort to establish the relationship between composition and structure of the silicates and their behavior with salt solutions.

*The concentration of NaCl necessary to form “absorbates”  
in the soil*

It is probable that as the soil silicates become more highly saturated with sodium, they will hydrolyze the more readily upon subsequent removal of the excess of salt. It was decided to determine to what degree the saturation must be carried before alkalinity could be produced.

For this study large quantities of soil 430 were placed on Büchner funnels and leached with NaCl solutions of various concentrations. After the treatment, the soil was air dried, pulverized, and placed in percolation tubes. The percolations proceeded under constant and equal heads of distilled water, as previously described. The results are tabulated in table 12.

TABLE 12  
EFFECT OF CONCENTRATION OF NaCl ON THE FORMATION OF “ABSORBATE”\*

Normality NaCl used	P.P.M.Na.	cc. N/10 acid required to neutralize one liter of percolate								
		June 11	June 13	June 14	June 18	June 21	June 24	July 5	July 19	July 26
.001	23	0	0	0	0	0	0	0	0	0
.01	230	no perc.	no perc.	no perc.	0	5.0	10	20	30	50
0.1	2,300	0	no perc.	Alk.	50	70.0	0†	0†	0†	no perc.
1.0	23,000	no perc.	0	0	130	350	350	420	0†	0†
4.0	92,000	0	0	0	10	120.0	0†	112	40	50

\* Series started June 10.  
† Reaction between iron rust and NaCl believed to have resulted in the formation of FeCl<sub>3</sub> and H<sup>+</sup> by hydrolysis.



It is clear from the table that a solution of NaCl containing 23 p.p.m. of sodium is not sufficient to produce an appreciable amount of sodium "absorbate" in this soil, but that a concentration of 2300 p.p.m. has a pronounced effect. The action of the normal solution (23,000 p.p.m.) is very notable, as shown in tables 5 and 6. The effect of 4 N NaCl is not easily understood. Its effect in producing alkalinity, as shown here, is considerably less than that of N/1 solution, but whether this was due to the difficulty in removing the large excess of NaCl or to a greater action on the iron rust accidentally introduced into the experiment, or to other causes, is not evident. Theoretically, 4 N NaCl should be more effective than N/1 NaCl, but it is not certain that other complicating factors were absent. This apparent anomaly should be investigated further.

The results of this experiment harmonize with many observations made in the field. Irrigation waters containing small amounts of sodium salts have often been used throughout the west without the production of alkalinity or undesirable physical conditions. With water containing more than 200 parts per million of sodium, however, injurious effects have been observed, which in certain instances, have developed in a relatively brief period. Accumulations of sodium salts in soils are frequently found, sufficient to give solutions of high concentration with rain water. The formation of hydrolyzable "absorbates," therefore, may readily take place in nature. Since the necessary concentration of sodium salts is low, it is possible that sodium carbonate may be formed wherever neutral salts occur. The limitation of the process appears to lie only in the complete saturation and subsequent complete hydrolysis of the silicates involved, but before this stage is reached the physical properties of the soil will become seriously deteriorated.

*The extent to which the excess of NaCl must be removed  
before alkalinity develops*

In the preceding experiments it was necessary to remove a part of the electrolyte from the salt-saturated soil before alkaline percolates were obtained. It has been observed many times in this laboratory, however, that dark-colored percolates and an impervious soil have resulted when only a small fraction of the total salt has been removed. Determination of the equilibria between the sodium of the newly formed "absorbates" and the sodium-ions in the solution surrounding

the solid particles should indicate the extent to which leaching must take place before hydrolysis is possible.

As water percolates downward through a column of soil it may leach out practically all of the salts from the upper layers, thus affording opportunity for hydrolysis and swelling in the upper portion of the column, but under these conditions the exact concentration of salt at the point where hydrolysis begins cannot be determined easily.

As a means of studying this point, soil 430' was first saturated with N/1 NaCl solution, air dried, pulverized, and placed in percolation tubes. Solutions of NaCl of various concentrations, ranging from slightly less than N/1 to N/1000 were then used instead of pure water. It is evident that the concentration of NaCl could not fall below that of the percolant, at any point in the soil. The results are shown in table 13.

TABLE 13

EFFECT OF CONCENTRATION OF NaCl ON THE HYDROLYSIS OF SOIL SATURATED WITH NORMAL NaCl SOLUTION

Percolant °	cc. N/10 acid per liter of percolate (Phenolph.)							
	June 11	June 13	June 14	June 18	June 21	June 24	July 5	July 19
H <sub>2</sub> O.....		0	0	130	350	350	420	0
0.01 N NaCl.....		34	60	20	Trace	0	0	0
0.1 N NaCl.....		40	40	8.0	0	0	0	0
0.5 N NaCl.....	Alk.	60	0	0	0	0	0	16
0.8 N NaCl.....	0	0	0	0	0	0		

The data show that the “absorbates” formed by a N/1 NaCl solution may hydrolyze to a certain extent in 0.5 N NaCl solution, but not in a concentration of 0.8 N NaCl. The hydrolysis became more marked as the concentration was reduced to 0.1 N and 0.01 N, but even then the extent of the hydrolysis was much less than with pure water. It would appear, therefore, that the more thorough the leaching the greater will be the formation of sodium carbonate.\*

*The equilibrium between sodium and calcium in  
“absorbate” formation*

To study the equilibrium between sodium and calcium in “absorbate” formation, portions of soil 430 were leached with solutions

\* Preliminary experiments made by E. E. Thomas indicate that prolonged leaching of semi-arid soils with dilute solutions of neutral sodium salts alone may result in the production of distinctly alkaline percolates.



containing a definite amount of NaCl, but variable amounts of CaCl<sub>2</sub>. The soil was then dried and pure water percolated through the soil columns as previously described. The results submitted in table 14 indicate that concentrations of CaCl<sub>2</sub> ranging from 0.001 N to 0.01 N are insufficient to prevent the action of N/1 NaCl; that 0.1 N solution of CaCl<sub>2</sub> effectively prevents it; while 0.001 N CaCl<sub>2</sub> solution was apparently without effect.

TABLE 14

EFFECT OF THE CONCENTRATION OF CALCIUM ON THE FORMATION OF SODIUM "ABSORBATES"

Original solution	cc. N/10 acid required to neutralize 1 liter of percolate (Phenolph.)								
	June 8	June 10	June 11	June 13	June 14	June 18	June 21	June 24	July 5
N/1 NaCl.....	no perc.	no perc.	no perc.	0	0	130	350	350	420
N/1 NaCl 0.001 N CaCl <sub>2</sub> ....	Alk.	134	170	215	300	400	.....	.....	.....
N/1 NaCl 0.01 N CaCl <sub>2</sub> .....	no perc.	no perc.	no perc.	0	0	5	50	100	100
N/1 NaCl 0.1 N CaCl <sub>2</sub> .....	no perc.	no perc.	no perc.	0	0	0	0	0	0

The effect of calcium ions upon the hydrolysis of sodium "absorbates"

For this study soil 430 was saturated with N/1 sodium chlorid as before, but instead of using pure water as the percolant, solutions of CaCl<sub>2</sub> of varying concentrations were employed. The data are shown in table 15.

TABLE 15

EFFECT OF CaCl<sub>2</sub> ON THE HYDROLYSIS OF SODIUM "ABSORBATES"

Percolant	cc. N/10 acid required to neutralize 1 liter of percolate (Phenolph.)								
	June 8	June 10	June 11	June 13	June 14	June 18	June 21	June 24	July 5
H <sub>2</sub> O.....	no perc.	no perc.	no perc.	0	0	130	350	350	420
N/1000 CaCl <sub>2</sub> .....	Alk.	0	17.5	240	180	100*	180*	230	.....
N/100 CaCl <sub>2</sub> .....	0	0	7.5	140	250	270*	210*	325	.....
N/10 CaCl <sub>2</sub> .....	0	0*	0†	0†	0†	0†	0†	0†	.....

\* Dark-colored percolate.

† Solution high in Ca.

The results show that a solution N/10 with respect to calcium (2000 p.p.m.) prevents the hydrolysis of sodium "absorbates" formed in this soil by N/1 sodium chlorid; but that N/1000 and N/100 calcium solutions are little more effective than pure water. These results indicate that in reclaiming soils which are highly saturated with sodium, the presence of considerable calcium in the irrigation water will aid in preventing their deterioration into alkaline lands.

The action of calcium-ions in preventing the hydrolysis of sodium "absorbates" is probably complex. First, calcium replaces sodium from the silicates forming calcium "absorbates." Second, calcium-ions exert a coagulating effect upon the colloids and thereby prevent their solution and hydrolysis. Third, any carbonate alkalinity produced by the hydrolysis of sodium "absorbates" would be precipitated as calcium carbonate.

### *The development of alkalinity in natural saline soils*

The conversion of natural saline lands into alkaline lands is reported to have been of great historical interest. Gedroits explains the relationship between these two types of soil and emphasizes that the one must inevitably result from the other, unless calcium salts are used in conjunction with leaching in the reclamation of such lands. Dominicus cites instances of the deterioration of vast areas of originally fertile soil, suggesting that they first became saline, then alkaline, and finally impoverished of all fine material by erosion, with resultant sterility. Laboratory confirmation of this view has not been reported, however.

A fine sandy loam soil, No. 887, was used for this purpose. This soil, taken from the Kearney Vineyard, Fresno County, California, contained slightly less than 1.0 per cent total soluble salts, but a 1:5 water extract gave no phenolphthalein alkalinity. A column of the untreated soil was subjected to percolation under a constant head of pure water. The first portions of the percolate were not alkaline to phenolphthalein, just as with the soils artificially treated with sodium chlorid. However, alkalinity soon developed in the percolate. The final percolate required 20 cc. N/10 acid to neutralize 1 liter to phenolphthalein; and 160 cc. for the methyl orange titration. The soil was finally removed from the percolator and extracted with five times its weight of carbon-dioxide-free water. The filtrate, after



passing through a Pasteur-Chamberland tube, gave a faint but noticeable alkaline reaction to phenolphthalein.

This experiment, therefore, affords an example of the conversion of a saline, non-alkaline soil into an alkaline soil, manifesting the physical characteristics of a "black-alkali" soil, containing residual sodium carbonate and yielding a distinctly alkaline percolate.

An interesting confirmation of this point was noted in unpublished experiments by A. R. C. Haas. He grew young orange trees in tanks of soil from the Citrus Experiment Station farm. The trees were irrigated for a time with dilute solutions of sodium chlorid. Later, an attempt was made to remove the excess of salt by leaching with distilled water. The drainage water was found to be distinctly alkaline to phenolphthalein. Continued leaching with distilled water ultimately gave percolates free from phenolphthalein alkalinity. Portions of the soil remaining in the tanks were later extracted with water in the usual 1:5 ratio and extracts with pH-values of 7.0 were obtained. In our work it has been noted that soils yielding very markedly alkaline percolates do not always give alkaline extracts when shaken with an excess of water. How a soil can yield a percolate containing 3 per cent sodium carbonate, for instance, and yet contain no determinable amount of residual alkalinity, is not clear.

#### GENERAL DISCUSSION

Previously two different explanations have been given regarding the origin of sodium carbonate in arid regions. One of these traces it directly to the weathering of igneous and metamorphic rocks; the other to the interaction of neutral sodium salts and calcium carbonate. According to the former view the soluble carbonates brought into solution by the action of meteoric waters tend to accumulate in places where the drainage and other conditions are favorable for their deposition. According to the latter, sodium salts reacting with calcium carbonate give rise to sodium carbonate and a soluble calcium salt. The fact that this latter reaction is capable of taking place has been known since the time of Berthollet<sup>2</sup> and has been periodically investigated down to the present time. Despite the experimental work the kinetics of the reaction have not been thoroughly established. The difficulty seems to lie in the necessity for the separation of the products of the reaction.

A third origin of sodium carbonate, discussed in this paper, has not been generally recognized. This, as set forth above, consists in the more or less complete saturation of the soil by neutral sodium salts, and in the subsequent hydrolysis of the sodium-silicate compounds thus formed. The conditions most favorable for this process are thorough and complete saturation of the soil with sodium and subsequent leaching. The extent to which the process may take place depends upon the nature of the soil, those containing much clay, silt, and abundant quantities of altered and secondary minerals, probably being the more reactive. Ideal conditions for this reaction are probably the exception in nature; but the above experiments show that small but not totally negligible amounts of  $\text{Na}_2\text{CO}_3$  may be formed under less favorable conditions. Where the conditions are decidedly favorable, large amounts of soluble alkaline compounds may eventually be carried down into the ground water or transported by seepage.

This reaction has been shown to be a consequence of basic exchanges between soil silicates and saline solutions. By essentially chemical processes, probably due to differences in their respective electrolytic solution potentials, sodium has been shown to replace a part of the calcium from the soil silicates readily and rapidly. When the concentration of sodium in solution is high and the calcium set free can be readily removed, a relatively high degree of saturation with sodium may occur. The physical properties of the new sodium-silicate combinations are different from those of the original calcium-silicate compounds, and are so pronounced as to alter profoundly the characteristics of the entire soil mass.

The sodium "absorbates," so-called, are much less stable than the corresponding calcium compounds. They are probably colloidal in nature, but this has not been established and is not a necessary assumption for an explanation of their behavior. Their important property is that of slowly hydrolyzing in the absence of strong electrolytes. Hydrolytic equilibrium is not rapidly established in nature because the hydroxyl-ions resulting from hydrolysis are readily removed by the  $\text{CO}_2$  in the soil atmosphere. This favors the decomposition of the "absorbates" and the development of alkalinity. Calcium carbonate may also favor the removal of the hydroxyl-ions.

The other product of the hydrolysis, the silicate complex, shows a tendency to be present in a colloidal state. This, as is well recog-



nized, may be an important factor favoring slow hydrolysis. Furthermore, reactions between the soluble hydrolytic products and the colloid probably favor a high degree of dispersion and account for the marked change in the physical properties of the soil. These striking changes in the physical properties of the soils were always observed associated or coincident with the development of alkalinity, and constitute one of the most important aspects of the phenomenon. The characteristic hardness and impermeability of many alkaline soils may be ascribed to the presence of sodium "absorbates," or the products of their hydrolysis.

Lessons may be drawn from these studies as to the proper treatment of saline soils. Leaching alone may be expected to lower the concentration of soluble salts, but in some cases at least, a deterioration of the land may be expected, partially to offset this advantage. In such cases some means must be provided to prevent the hydrolysis of the sodium "absorbates" with their attendant deflocculation.

Any means of preventing hydrolysis during and after the removal of the soluble salts, such as the addition of soluble aluminum, ferrous and ferric salts, acids and organic matter, may be expected to be beneficial. There may be some objection to the use of some of these substances, however, since the soil may be left in an unfavorable condition for crop growth. Moreover, none of these materials can correct the fundamental mischief caused by the original sodium salts, i.e., they cannot restore the calcium to its original position in the active soil silicates. The importance of calcium in the silicate-complexes of the soil in maintaining a desirable physical and chemical medium for plant growth can hardly be over-emphasized. Soluble calcium salts should not, therefore, be ignored in the practical reclamation of saline lands.

### SUMMARY

(1) When semi-arid soils are brought to equilibrium with a sodium salt the combined calcium, magnesium, and potassium passing into solution are chemically equivalent to the sodium fixed, and the total of these ions is an exponential function of the concentration of the sodium salt remaining in solution. It was found that the sodium fixed is an exponential function of the concentration of the sodium salt remaining in solution at equilibrium.

(2) These relationships conform to the general type of Freundlich's adsorption curves and equations. The reactions are considered to be primarily chemical in nature.

(3) Upon treatment with a sodium salt, the soil undergoes a change reciprocal to that occurring in the solution. While the solution becomes enriched with calcium, the soil becomes impoverished of that element. The solid particles, therefore, become relatively richer in sodium.

(4) The new sodium-silicate complexes formed by the action of sodium salts are less stable, more soluble, and more easily hydrolyzable than the corresponding calcium complexes. The properties of these sodium compounds are so pronounced as to modify profoundly the physical properties of the entire soil mass.

(5) The effects of saturating a soil with sodium are most pronounced upon subsequent leaching with relatively pure water. The soil then swells, becomes impervious and yields a dark-colored and frequently strongly alkaline percolate. These reactions are considered to be important in the formation of sodium carbonate in nature.

(6) The formation of alkalinity is dependent upon the tendency of the sodium-silicate compounds to hydrolyze in the absence of too strong concentrations of electrolytes, with the resultant formation of sodium hydroxide. The NaOH is readily converted into  $\text{Na}_2\text{CO}_3$  by the  $\text{CO}_2$  of the soil solution.

(7) Some of the products of the hydrolysis have a tendency to assume the colloidal state, which, probably accentuated and maintained by the hydroxyl-ions, accounts for the deflocculated condition of the soil. Calcium carbonate was shown not to be essential for the formation of  $\text{Na}_2\text{CO}_3$  by this reaction.

(8) Certain soils from humid regions, one of which was distinctly acid, reacted similarly but not as markedly as semi-arid soils.

(9) Alkalinity was also developed by percolating water through columns of granites and several pure mineral silicates which had previously been treated with sodium chlorid.

(10) With the soil studied N/100 solution of sodium chlorid was found to be of sufficient concentration to form hydrolyzable compounds; N/10 and N/1 solutions were still more potent in this respect, while N/1000 solutions had no apparent effect.



(11) It was found that the concentration of the saturating solution of sodium chlorid must be reduced about one-half before alkalinity develops, but that lowering the concentration still further greatly increases the alkalinity. The greatest amount of alkalinity was obtained when the sodium-saturated soil was leached with pure water.

(12) Soluble calcium salts, if present in sufficient concentration, will prevent the formation of hydrolyzable sodium compounds in the soil. With a mixed solution of normal sodium chlorid the addition of  $\text{CaCl}_2$  equivalent to N/1000 had no effect; N/100  $\text{CaCl}_2$  reduced the amount, while N/10  $\text{CaCl}_2$  completely prevented the reaction.

(13) Calcium-ions tend to prevent the hydrolysis of sodium absorption compounds once they have been formed. N/10  $\text{CaCl}_2$  solution completely prevented the hydrolysis in soil 430, while N/100 and N/1000  $\text{CaCl}_2$  solution were little more effective than pure water.

(14) An example is given of the conversion of a natural saline soil into an alkaline soil, and of the development of alkalinity in a soil by mild treatment with sodium chlorid and subsequent leaching with pure water.

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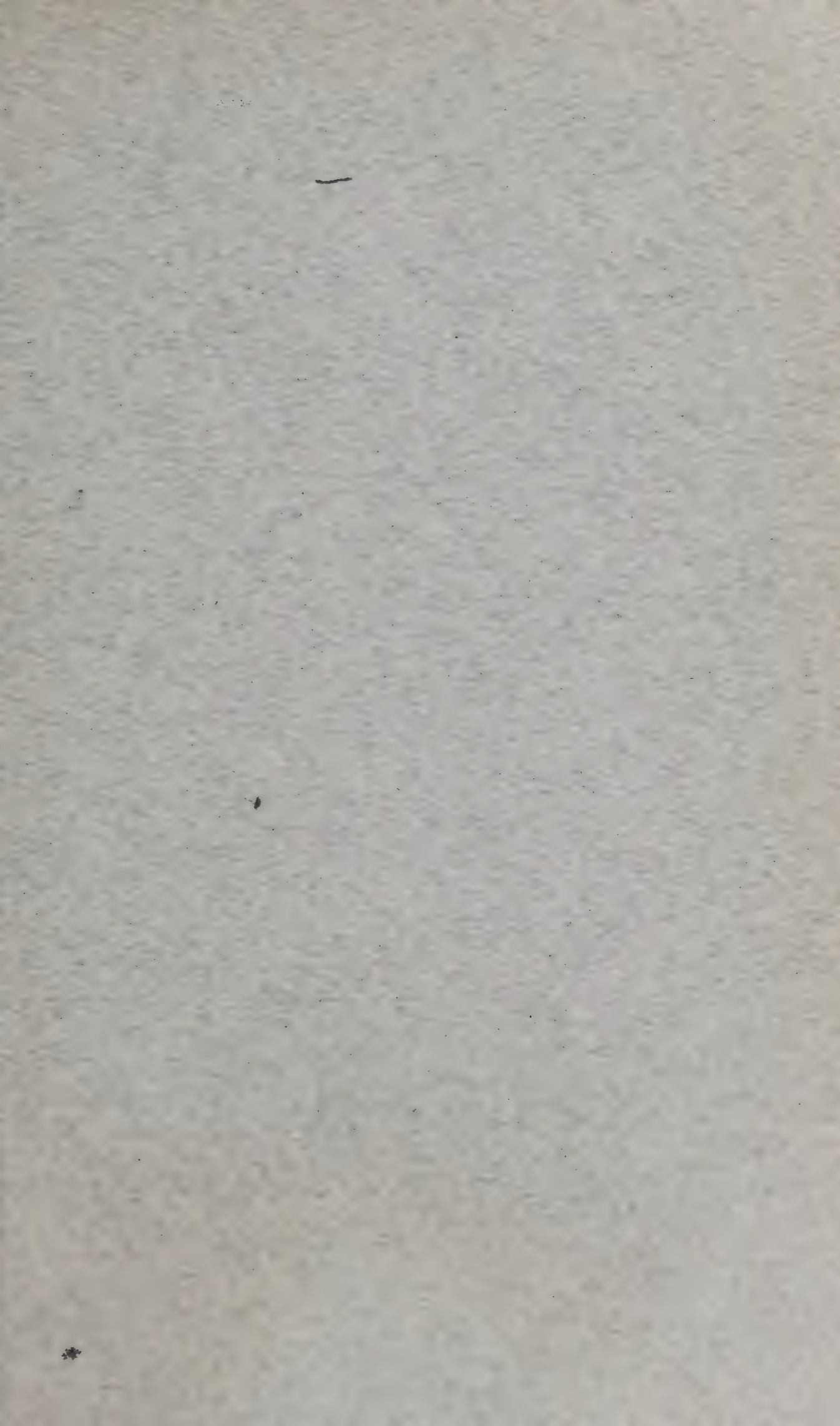
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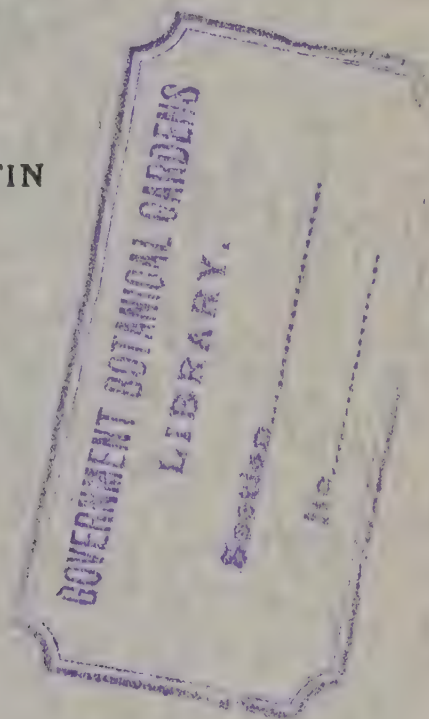
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TECHNICAL PAPER No. 8

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(Contribution from the Division of Plant Nutrition, University of California)

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INTRODUCTION

In arid regions various types of injury to plants have been associated with the presence of alkali\* salts in the soil. While many observations have been made in the field for the purpose of determining the tolerance of different plants to alkali conditions, comparatively few controlled experiments have been carried out with intent to study the effect of an alkali soil or solution on the chemical system of the plant. Information of this character, however, is essential for an understanding of the relation of the plant to the medium in which it grows. Only by means of intensive studies made on the plant itself will it be possible to reach definite conclusions concerning the nature of the injury produced by different salts or to explain the varying responses of different plants growing in the same soil or solution. A systematic investigation of these questions is being attempted in this laboratory and certain data bearing on one phase of the work are reported in the present article.

Previous studies of the absorption of inorganic elements by barley plants suggested that it would be of considerable interest to ascertain how sodium salts influence the absorption of important ions from

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\* The term 'alkali' is used in its general and customary sense, but it is well to note that the expression is technically inaccurate. As our knowledge advances, it will be desirable to substitute other more exact expressions, such as salinity, alkalinity, and appropriate sub-classifications.

culture\* solutions. Both sand and solution cultures were employed in the course of the investigations. In one group of experiments, the absorption studies were conducted for very short periods and the solutions were analyzed. In another group the plants were grown for longer periods in the solutions under examination and were themselves analyzed. In one instance, the plants were grown to complete maturity in sand cultures.

Data on the intake of ions by the plant are often of value to the investigator interested in soil and plant relations, but it is evident that certain limitations may have to be placed on the interpretation of such data. For example, Osterhout<sup>1</sup> and Brooks<sup>2</sup> have pointed out that the rate of absorption of an ion is not necessarily a measure of cell permeability. Various reactions occurring in the cell, on surfaces, or in intercellular spaces may cause the ion to be removed from the influence of diffusion equilibria, with the result that considerable absorption may take place even when the cell possesses a low degree of permeability. It may be noted that a somewhat different conception of permeability has been presented by Stiles and Jorgensen.<sup>3</sup> In the present investigation no attempt is made to interpret the results in terms of cell permeability.

#### EFFECT OF SALTS ON ABSORPTION OF IONS

The technique of the first experiments described consisted in growing large numbers of barley plants during a period of two to three weeks in a complete culture solution† and then dividing the cultures into uniform sets. Each set consisted of either 49 or 98 plants, duplicate sets being used in a number of experiments. After rinsing the roots with distilled water, the plants were transferred to the various solutions that were to be examined. The culture vessels were tumblers of about 110 c.c. capacity, each provided with a cork stopper bored with seven holes. At the conclusion of the experiment the solutions were removed from the plants, made up to volume, and analyzed. Water was added as required during the period of the

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\* The expressions 'culture solutions' and 'nutrient solutions' are used interchangeably in this paper, according to custom, but the latter term cannot be considered to be strictly accurate.

† Similar to that used in previous nutrition studies. Composition given in table 1.



TABLE 1.—EFFECT OF NaCl AND Na<sub>2</sub>SO<sub>4</sub> ON THE ABSORPTION OF NUTRIENTS IN SOLUTION CULTURE

Description of Solution	Salt added		K R.V.	Ca R.V.	Mg R.V.	NO <sub>3</sub> R.V.	(H <sub>2</sub> ) PO <sub>4</sub> R.V.	SO <sub>4</sub> R.V.	H <sub>2</sub> O Absorbed c.c.
	R.V.	P.P.M. Na							
<i>Experiment A—3-Day Period</i> (October 29—November 1)									
Composition of nutrient solution.....			4.35	8.55	4.10	11.2	1.16	4.12	
Absorption from nutrient solution.....			3.43	1.15	.33	7.5	.29	.33	900
* Absorption from nutrient solution plus NaCl.....	34.8	800	2.12	.90	.25	7.7	.28	.44	800
* Absorption from nutrient solution plus Na <sub>2</sub> SO <sub>4</sub> .....	34.8	800	2.23	.85	.14	8.7	.27		900
<i>Experiment B—3-Day Period</i> (July 20-23)									
Composition of nutrient solution.....			4.08	8.97	5.42	11.9	1.30		
Absorption from nutrient solution.....			3.22	1.80	.82	7.5	.36		1180
Absorption from nutrient solution plus NaCl.....	87.0	2000	.56	.70	.74	5.9	.17		810
<i>Experiment C—7-Day Period</i> (March 30—April 7)									
Composition of nutrient solution.....			4.76	9.20	3.54	11.2	1.25		
Absorption from nutrient solution.....			4.50	4.29	.74	11.0	.95		1860
Absorption from nutrient solution plus NaCl.....	87.0	2000	3.07	3.74	.99	11.0	.78		1760

\* Average of duplicate sets. Maximum difference in absorption R.V. K .3, Ca .15, Mg .24, NO<sub>3</sub> .48, PO<sub>4</sub> .06.

experiment, and the total absorption noted. It was hoped that the procedure just outlined would permit comparisons to be made of the intake of ions by similar plant systems, in which differences in growth and cumulative effects would be minimized.

The results obtained, calculated in terms of reaction values,\* are set forth in table 1. It is evident that in all the experiments a marked and consistent depression of the absorption of potassium occurred. The intake of calcium was also depressed, but not so consistently. The values for the absorption of magnesium are small, and any interpretation of the results uncertain. In two of the cases no effect was produced on the  $\text{NO}_3$  ion, and in the other case the percentage effect was much less than for several other ions. The absorption of  $\text{PO}_4$  was depressed markedly in one experiment. The main suggestion gained from these studies is that the absorption of potassium, and possibly of other ions, is depressed when a relatively high concentration of sodium salts is present in the solution. The reduction in water intake was not sufficient to account for all of the effects noted. It may be added that in other experiments similarly conducted in which calcium salts were used alone and in the presence of sodium chlorid, the absorption of calcium was depressed very significantly. When calcium was present below a certain concentration, the presence of sodium salt caused a loss of calcium from the plant, while at higher concentrations absorption of calcium occurred. The loss of calcium to the solution may, of course, be attributed to leaching from dead cells, but it is probable that a chemical displacement of calcium also occurs. In every case sodium and chlorin were removed from solution in appreciable quantities.

In continuation of these studies barley plants were grown for longer periods in culture solutions containing sodium salts. In two cases the plants were first grown in the unmodified culture solution and then transferred to solutions containing the sodium salts. In one experiment the plants were grown continuously in culture solutions plus sodium chlorid or sodium sulfate. In the solution culture experiments a large number of plants was grown in each solution. The details of these experiments and analyses of the plants obtained are given in tables 2 and 3. It is quite apparent that in every instance the presence of sodium salts in the culture solution caused a marked and significant

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\*  $\frac{\text{Valence}}{\text{atm. wt.}} \times \text{p.p.m.} = \text{milliequivalents.}$



TABLE 2.—COMPOSITION OF THE TOPS AND ROOTS OF PLANTS GROWN IN NUTRIENT SOLUTIONS, AND IN NUTRIENT SOLUTIONS CONTAINING SALTS

Description of nutrient solution	Salt added		Composition of tops of plants (Air dry basis) Per cent						Composition of roots of plants (Air dry basis) Per cent						Per cent of total Cl absorbed	Total weight of tops green	Total weight of tops dry	Total weight of roots dry	No. of plants
	R.V.	P.P.XI.	N	PO <sub>4</sub>	Ca	Mg	K	Cl	N	PO <sub>4</sub>	Ca	Mg	K	Cl					
Nutrient.....			3.15	1.96	1.55	.50	3.76	<i>Experiment A</i>	2.45	2.76	1.33	.25	1.72			920	114	26.9	35
Nutrient plus NaCl	17.1	1000	3.10	2.06	1.22	.37	3.87	5.28	2.30	3.02	1.25	.19	1.90	4.70	68.0	1150	109	25.6	36
Nutrient plus NaCl	85.5	5000	3.06	2.20	.72	.25	2.60	7.78	2.70	3.53	.64	.17	1.96	6.20	25.0	780	140	24.5	34
Nutrient.....			5.24	2.42	1.23		6.11	<i>Experiment B</i>	4.48	2.26	1.32		5.06			232	25	7	140
Nutrient plus NaCl	85.5	5000	4.42	2.58	.58		3.85		4.06	3.38	.43		2.90			177	21	7	140

*Experiment A.*—Plants grown in nutrient solution for 1 month, then changed to solutions described above and grown 4 weeks. (September 17–October 14.)

*Experiment B.*—Plants grown 1 week in nutrient solutions, and then 4 weeks in solutions as described, with frequent changes of solution. (April 14–May 12.)

TABLE 3.—COMPOSITION OF PLANTS GROWN IN SAND CULTURES IN NUTRIENT SOLUTIONS WITH AND WITHOUT ADDITION OF SODIUM SALTS

	Description of nutrient solution		Freezing point depression of solution, degrees C.	Percentage of water free material								
	Salt added			K	Ca	Mg	Na	N	PO <sub>4</sub>	Cl	SO <sub>4</sub>	Total S
	R.V.	P.P.M.										
<i>Nutrient solution</i> .....			.057	1.52	.89	.32		.44	.26		1.08	.55
Stems and leaves.....				.47	.06	.12		1.54	1.15		.09	
Grain.....				1.31	.61	.35		.50	.38		.59	
Chaff.....												
<i>Nutrient solution + NaCl</i> .....	51.3	3000	.237	1.78	.71	.23	1.65	.47	.25	2.58		.49
Stems and leaves.....				.43	.07	.13		1.69	1.03	.17		
Grain.....				1.87	.79	.28		.55	.30	.98		
Chaff.....												
<i>Nutrient solution + NaCl</i> .....	102.6	6000	.418	2.02	.63	.21	2.58	.63	.25	3.59		.49
Stems and leaves.....				.45	.05	.10		1.97	1.02	.25		
Grain.....				2.25	.53	.27		.61	.29	1.69		
Chaff.....												
<i>Nutrient solution + Na<sub>2</sub>SO<sub>4</sub></i> .....	70.4	5000	.223	1.16	.49	.16	1.43	.46	.34		1.78	.78
Stems and leaves.....				.37	.07	.15		1.69	1.05		.14	
Grain.....				1.39	.43	.23		.54	.32		1.14	
Chaff.....												
<i>Nutrient Solution + Na<sub>2</sub>SO<sub>4</sub></i> .....	140.8	10000	.374	.90	.48	.15	2.39	.45	.34		2.21	.97
Stems and leaves.....				.55	.05	.12		1.86	1.09		.12	
Grain.....				1.73	.46	.25		.61	.34		1.70	
Chaff.....												

Period of growth, May to September.



alteration in the composition of the plant tissue. In general, the percentages of cations were depressed, while relatively smaller effects are noted in the case of the anions. In several instances the percentages of nitrogen and phosphorus were increased to a greater or less extent. In the experiment in which plants were grown to maturity in solutions (sand cultures) containing sodium chlorid, some increase occurred in the percentage of potassium found in the stems and leaves and chaff. The total quantities of potassium absorbed on the average by a plant were similar, however, for the culture solution and for the culture solution plus sodium chlorid (table 4). Where there was a decreased yield of crop, the percentage of potassium increased correspondingly. It would seem, therefore, that under these circumstances the decreased rate of absorption of potassium did not depress the total intake of potassium when the entire period of growth was considered, as a large proportion of all of the potassium present in the solution was removed. This does not apply to calcium and magnesium, since these elements are also found in smaller percentages and total quantities in the mature plants. The plants grown in the solutions to which the sodium sulfate was added differ from the plants grown in the sodium chlorid solution in that the percentages in stems and leaves and the total quantities of potassium absorbed are decreased to a marked extent, especially in the case of the higher concentration of sulfate. A greater effect was also produced on the content of calcium and magnesium. No significant decreases are noted in the percentage of nitrogen and phosphorus in any portion of the plant. Marked differences in the composition of the grain are not evident, except a possible increase in the percentage of nitrogen.

The total equivalents of sodium present in the stems and leaves exceeded those of potassium, calcium, or magnesium, when sodium was present in the culture solution. Sodium also tended to cause a change in the relation of potassium to calcium by decreasing the proportion of the latter.

Very considerable percentages of chlorin and sodium are stored in the stems and leaves, and the grain itself contains appreciable quantities of chlorin. The previous solution culture experiments showed a still greater accumulation of chlorin (and presumably of sodium) in the plant tissue. The equivalent weight of sulfate

TABLE 4.—TOTAL QUANTITIES OF INORGANIC ELEMENTS ABSORBED BY PLANTS GROWING IN SAND CULTURES WITH AND WITHOUT ADDITION OF SODIUM SALTS  
(Average per Plant)

	Description of nutrient solution			Weight of dry material Gms.	K	Ca	-Mg	Na	N	PO <sub>4</sub>	Cl	SO <sub>4</sub> (in ash)	Total S (Calculated to SO <sub>4</sub> )
	Salt added		Freezing point depression of solution, degrees C.										
	R.V.	P.P.M.											
<i>Nutrient solution:</i>							(Gram equivalents x 1000)						
Stems and leaves.....			.057	6.63	2.59	2.94	1.73	.....	2.07	.18	.....	1.50	2.23
Grain.....				9.92	1.20	.29	.99	.....	10.94	1.20	.....	.10	
Chaff.....				1.50	.51	.45	.41	.....	.57	.06	.....	.19	
Total.....				18.05	4.30	3.68	3.13	.....	13.58	1.44	.....	1.79	
<i>Nutrient solution plus NaCl.....</i>	51.3	3000	.237										
Stems and leaves.....				5.75	2.64	2.05	1.07	4.13	1.93	.15	4.17	.....	1.74
Grain.....				11.63	1.28	.40	1.23	.....	14.01	1.26	.56	.....	
Chaff.....				1.63	.79	.65	.33	.....	.64	.05	.45	.....	
Total.....				19.01	4.71	3.10	2.63	.....	16.59	1.46	5.18	.....	
<i>Nutrient solution plus NaCl.....</i>	102.6	6000	.418										
Stems and leaves.....				4.31	2.23	1.35	.74	4.83	1.93	.12	4.37	.....	1.30
Grain.....				9.69	1.13	.25	.82	.....	13.66	1.04	.68	.....	
Chaff.....				1.31	.74	.35	.33	.....	.57	.04	.62	.....	
Total.....				15.31	4.10	1.95	1.89	.....	16.16	1.20	5.67	.....	
<i>Nutrient solution plus Na<sub>2</sub>SO<sub>4</sub>.....</i>	70.4	5000	.223										
Stems and leaves.....				5.94	1.77	1.55	.74	3.70	2.00	.21	.....	2.20	2.85
Grain.....				9.75	.92	.35	1.15	.....	11.80	1.08	.....	.29	
Chaff.....				1.69	.61	.40	.33	.....	.64	.06	.....	.40	
Total.....				17.38	3.30	2.30	2.22	.....	14.44	1.35	.....	2.89	
<i>Nutrient solution plus Na<sub>2</sub>SO<sub>4</sub>.....</i>	140.8	10000	.374										
Stems and leaves.....				3.94	.92	.95	.49	4.09	1.29	.14	.....	1.81	2.36
Grains.....				7.00	.97	.20	.66	.....	9.29	.80	.....	.16	
Chaff.....				.94	.41	.20	.25	.....	.43	.03	.....	.33	
Total.....				11.88	2.30	1.35	1.40	.....	11.01	.97	.....	2.30	

16 to 24 plants used for each solution.

The number of plants does not warrant statistical treatment, but the large and consistent differences in composition, as shown above, indicate that variability does not invalidate the conclusions reached.



absorbed was decidedly less than that of chlorin. This relationship is especially brought out by a comparison of the figures representing the percentages removed of the total chlorin and of the total sulfate present in the solution, this value for the chlorin being from three to seven times that for the sulfate. The equivalent weight of sodium stored in the stems and leaves considerably exceeds that of the total sulfur. These facts are in accord with the results of other experiments carried out in this laboratory, to be described later, which indicate that the sulfate ion is absorbed by barley at a relatively slow rate, while the activity of chlorin in this regard is sometimes more nearly comparable to that of the nitrate ion.

With regard to the crop production (table 4), it may be noted that slight decreases in total weight of stems and leaves were brought about by the lower concentrations of salts, with more pronounced effects for the higher concentrations. For similar osmotic concentrations sodium chlorid and sodium sulfate produced similar decreases in yield. No decrease in the grain produced is noted, except in the case of the high sulfate solution. Considering the relatively small number of plants grown in the sand cultures, the decrease of yield with the lower concentrations of salt may not be significant. No certainly significant differences were noted in respect to height, number of heads, or of tillers. In general, the salts in the concentrations used did not cause visible injury to the plant other than the decrease in yield. The plants grown in the salt solutions matured a little earlier than those grown in the unmodified nutrient solution.

It may be stated here, incidentally, that other sand and solution culture experiments with barley do not indicate that sodium chlorid is definitely more toxic than the sulfate when equal osmotic concentrations are compared. The comparison of the salts on a percentage basis according to the usual custom would probably point to the greater toxicity of sodium chlorid, but from a physiological standpoint it appears that the comparisons should rather be made on the basis of osmotic concentrations or of equal concentrations of sodium. Observations on the relative toxicity of these salts when present in the soil introduce many complicating questions concerning the actual concentrations of the various ions in the soil solution. It will be recalled that Hilgard placed the relative toxicity of sodium chlorid and sulfate as five to one.

Several experiments were also carried out with cucumbers and cantaloupes, comparing the effects of sodium chlorid and sulfate solutions of equal osmotic concentrations. With this type of plants it was also found that sodium sulfate was not less toxic than sodium chlorid and in several instances, in fact, the former salt appeared to be even more toxic to these plants. The absorption of electrolytes by the plants was greater from the chlorid than from the sulfate solution under the experimental conditions. From other considerations it is probable that the relatively smaller absorption of ions from the sulfate solution was not simply the result of inhibited plant growth, but was,

TABLE 5.—EFFECT OF SODIUM SALTS ON ABSORPTION OF CALCIUM BY BEANS

Description of solution	Salt added		Dry of tops, Grms.	Ca %	Total Ca Grms.
	R.V.	P.P.M.			
Control.....			8.2	1.84	.151
Control + NaCl.....	17.1	1000	9.0	1.58	.142
Control + Na <sub>2</sub> SO <sub>4</sub> .....	17.1	1215	9.9	1.43	.142
Control + NaCl.....	51.3	3000	5.6	1.31	.073
Control + Na <sub>2</sub> SO <sub>4</sub> .....	51.3	3645	6.0	1.22	.073
Control + NaCl.....	85.5	5000	3.4	1.20	.041
Control + Na <sub>2</sub> SO <sub>4</sub> .....	85.5	6075	4.6	1.16	.053

Plants grown for 6 weeks in sand culture. 25 plants used for each solution.

Analyses averages of closely agreeing duplicate determinations.

in part, related to the slow rate of penetration of the sulphate ion. It might be suggested here that an increased rate of absorption of ions would tend toward the attainment of osmotic relations between solution and plant which would favor the absorption of water.

One experiment was also made with bean plants (small white) in sand culture. The results presented in table 5 show very appreciable decreases in the percentages of calcium contained in the stems and leaves when the plants were grown in solutions to which sodium chlorid or sulfate was added.

The next question considered pertained to the relative toxicity and the absorption of ions with a salt of a different type. Comparison was, therefore, made between solutions containing sodium salts and others containing calcium chlorid. These experiments with barley



indicated that for equal concentrations of chlorin and similar osmotic values calcium chlorid was more toxic than sodium chlorid at the higher concentrations. Special absorption studies were also made with single salt solutions of calcium chlorid to determine the relative rate of intake of calcium and of chlorin ions. It was found that, under the conditions of the experiment, the reacting weights of chlorin absorbed exceeded those of calcium. The ionic balance in the solution was restored by the excretion or formation of  $\text{HCO}_3$  ions, the reaction of the solution becoming practically neutral. The injury to the barley plants grown during the summer months in calcium chlorid solutions containing 5000 p.p.m. of calcium chlorid or more was very marked, whether the calcium chlorid was used alone or was added to a complete nutrient solution. The leaf injury appeared to be more pronounced than the root injury, at least in the first stages. From a few preliminary tests it appeared that the toxicity of magnesium chlorid was of similar type, but more intense. Sand culture experiments with calcium chlorid gave results similar to those obtained from solution cultures.

Since the toxicity of the calcium chlorid to barley was greater than might have been anticipated, some consideration was given to the possibility that impurities of a toxic nature were present in the salt used. Numerous tests were made for toxic elements which might conceivably have contaminated the salt during the processes of manufacture, but so far the results obtained do not indicate that the effects produced on the plant can be ascribed to this cause. It is not desired at present, however, to draw any final conclusions concerning the relative toxicity of various salts, since a systematic study of the question is now being made. Toxicity is intimately related to seasonal conditions, as will be pointed out later in the discussion. During winter the toxicity of calcium chlorid and of other salts has been found to be far less marked than during summer or spring. It should also be noted that statements concerning relative toxicities will vary according to the criteria used. Thus a study of the effect on the plant as a whole, after a sufficient exposure to the salt-containing solution, may not lead to the same conclusion as observations on root growth over a brief interval of time. This is particularly true when single salt solutions are used in determining root injury, as in the experiments of Kearney and Cameron.<sup>4</sup>

## EFFECT OF SALTS ON REACTION, BUFFER SYSTEM, AND OSMOTIC VALUES OF THE EXPRESSED PLANT SAP

Since 'alkali' salts were found to be readily absorbed by the plant, it was reasonable to suppose that certain corresponding changes might be produced in the composition or chemical constants of the expressed plant sap.\*

The following determinations were made on the plant juices in an attempt to ascertain what modifications were produced by the salts added to the culture solution: freezing point depression, hydrogen ion concentration, and buffer effect.

The technique of the culture experiments was simple, and consisted in growing a large number of plants (about 500) in shallow pans filled with a culture solution. The plants were supported on wooden frames fitting over the top of the pan and covered with wide-meshed mosquito netting lightly impregnated with paraffin. After the plants had been grown for several weeks in this way, the solutions were replaced by new supplies of culture solution to which were added the desired quantities of salts to be tested. In this way the effects of the salts on plants of similar development were compared. The period of contact with the salt solutions was comparatively brief, the intention being to produce only the first stages of injury. The plants were then removed from the solution and rinsed with distilled water; the roots and tops were separated, placed in jars, and then frozen in a refrigerator room at  $-15^{\circ}$  C. The material, after being frozen, was thawed quickly, the juice expressed with the aid of an ordinary screw press, and then rapidly filtered through paper pulp with the aid of suction. Determinations on the juices were made immediately after expression and filtration. It is well known that the technique and pressure employed affect the composition of plant juices. In these experiments the differences produced by the various treatments are frequently considerable and only comparative values are sought. It has been pointed out by Haas<sup>5</sup> that it is desirable to examine the different parts of the plants (such as stems, leaves, and petioles) separately. While this has not been done in the present work, the

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\* The sap (or juices) expressed from a plant cannot, of course, be assumed to be identical with the true cell sap.



nature of the experimental conditions renders it probable that the same relative proportions of the different parts were present in the comparable sets.

Three different types of plants were used in the studies on plant sap, barley (Beldi variety), peas (field), and pumpkins (Connecticut field). In one experiment barley and pumpkins were grown together

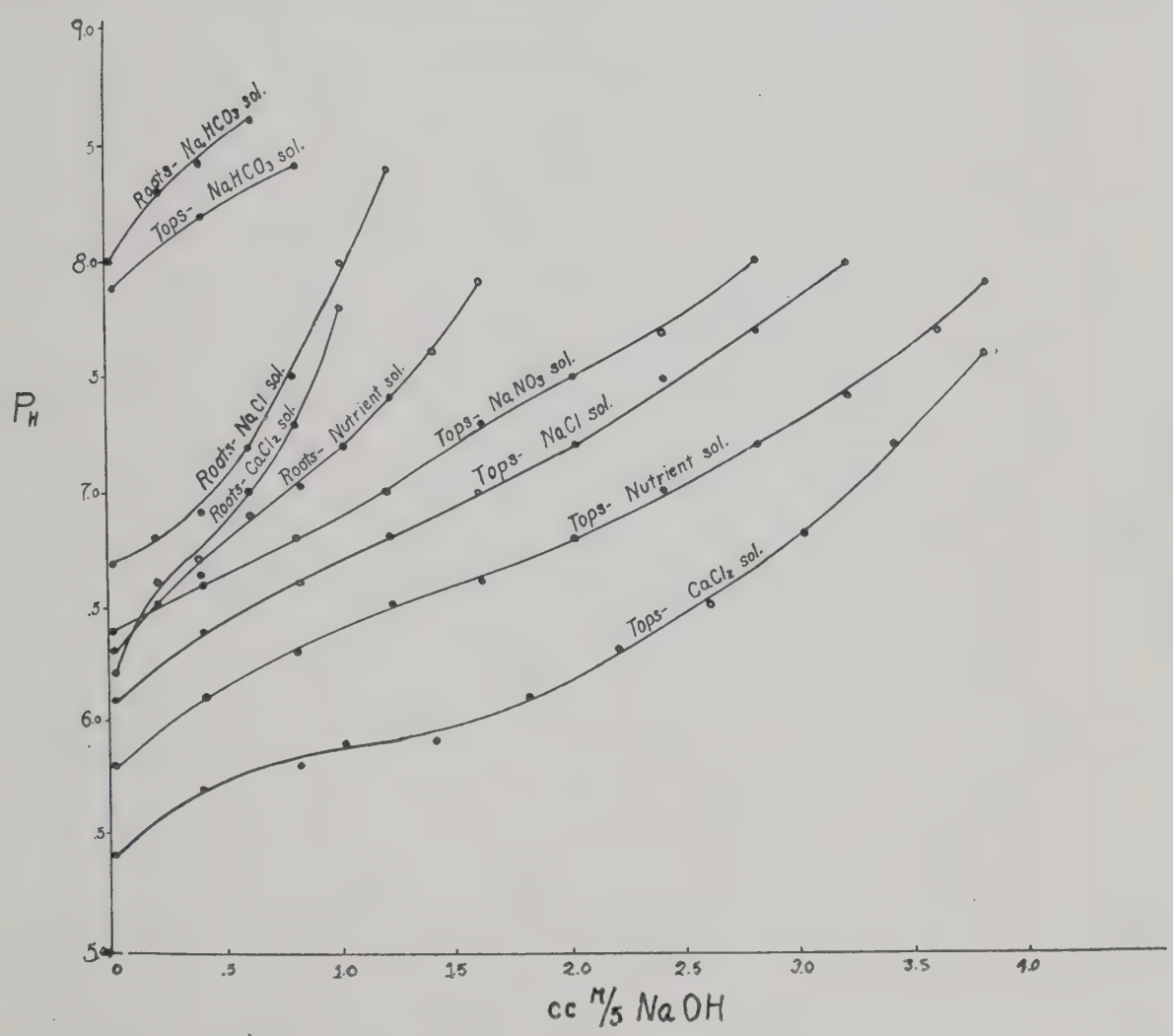


Fig. 1. Titration curves for sap (25 c.c.) expressed from barley plants grown in nutrient solution and in nutrient solutions with the addition of various salts. For details, see table 6, Experiment A.

in mixed culture, in order that the roots of both plants might always be in contact with the same solution.

Considering first the hydrogen ion concentration and titration values of the sap from the barley plants, it is observed that, in the sap expressed from the tops of the plants, the calcium chlorid solution depressed the pH value and increased the quantity of alkali required in the titration to a given pH. The sodium bicarbonate (fig. 1) brought the reaction of the sap to a distinctly alkaline point. Sodium nitrate

acted in the same direction, but to a less extent. The least change occurred in the case of the sodium chlorid solution, although a slight increase of pH and a decreased buffer effect was observed. The sap expressed from the roots grown in the sodium bicarbonate solution showed a decided increase of alkalinity and a decrease of titration value. A slightly increased pH value was produced by the sodium chlorid solution. Calcium chlorid, however, did not give rise to the same effect on the expressed root sap as it did on the sap from the tops. The change produced in the roots was small and consequently may fall within the limits of experimental error.

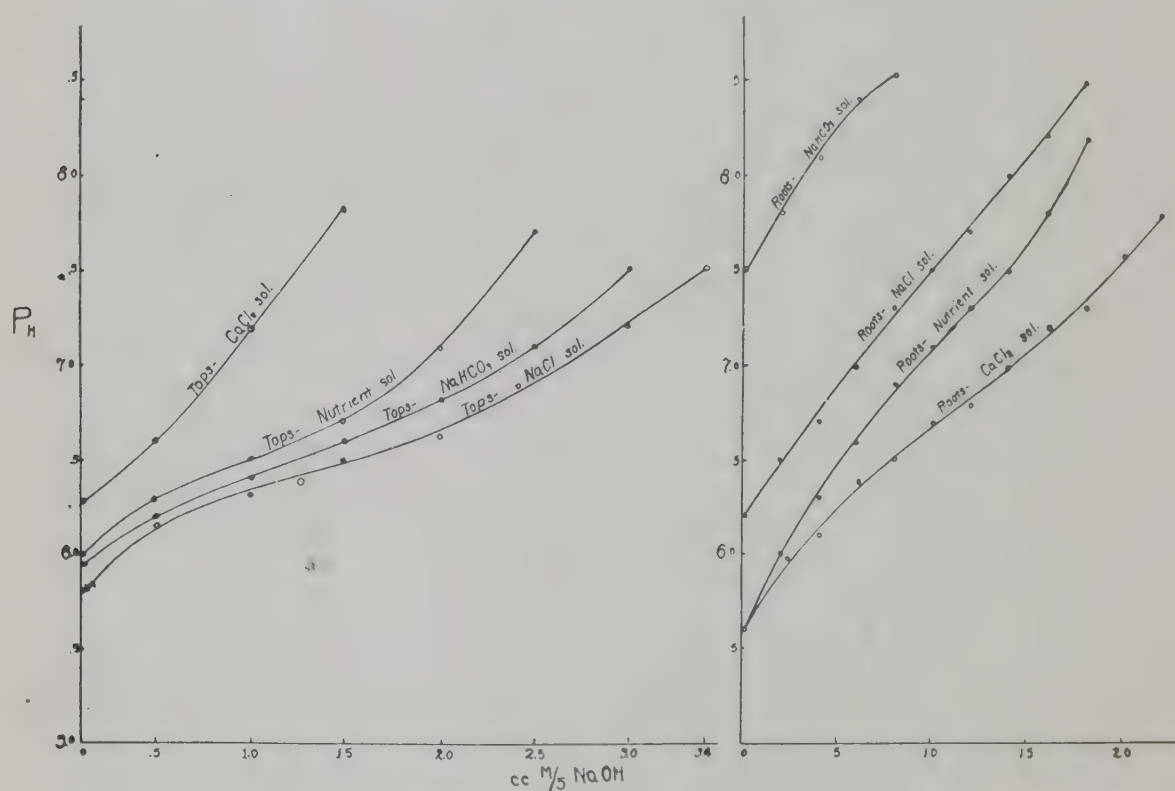


Fig. 2. Titration curves for sap (25 c.c.) expressed from pumpkin plants grown in nutrient solution, and in nutrient solutions with the addition of various salts. For details, see table 6, Experiment B.

Calcium chlorid caused the most marked injury to the plant as a whole under the given experimental conditions. The greatest injury to the roots occurred in the solutions to which sodium bicarbonate was added, but it is interesting to note that a similar, although much less extensive, injury to the roots was produced with sodium nitrate. In the solution containing sodium nitrate there was no increase of alkalinity over that of the culture solution. Nevertheless, the reaction of the expressed root sap was increased from pH 6.3 to pH 6.9, as indicated by colorimetric estimation.



The experiments with pumpkins showed, for the tops, a different set of relations in the expressed sap (fig. 2). The calcium chlorid curve for the tops was displaced to the alkaline side, instead of to the acid side as in the case of barley. Both the sodium bicarbonate and sodium chlorid curves were displaced slightly to the acid side. The root sap exhibited much the same behavior as that from barley, except that the calcium chlorid caused a definite increase in the acid reserve.

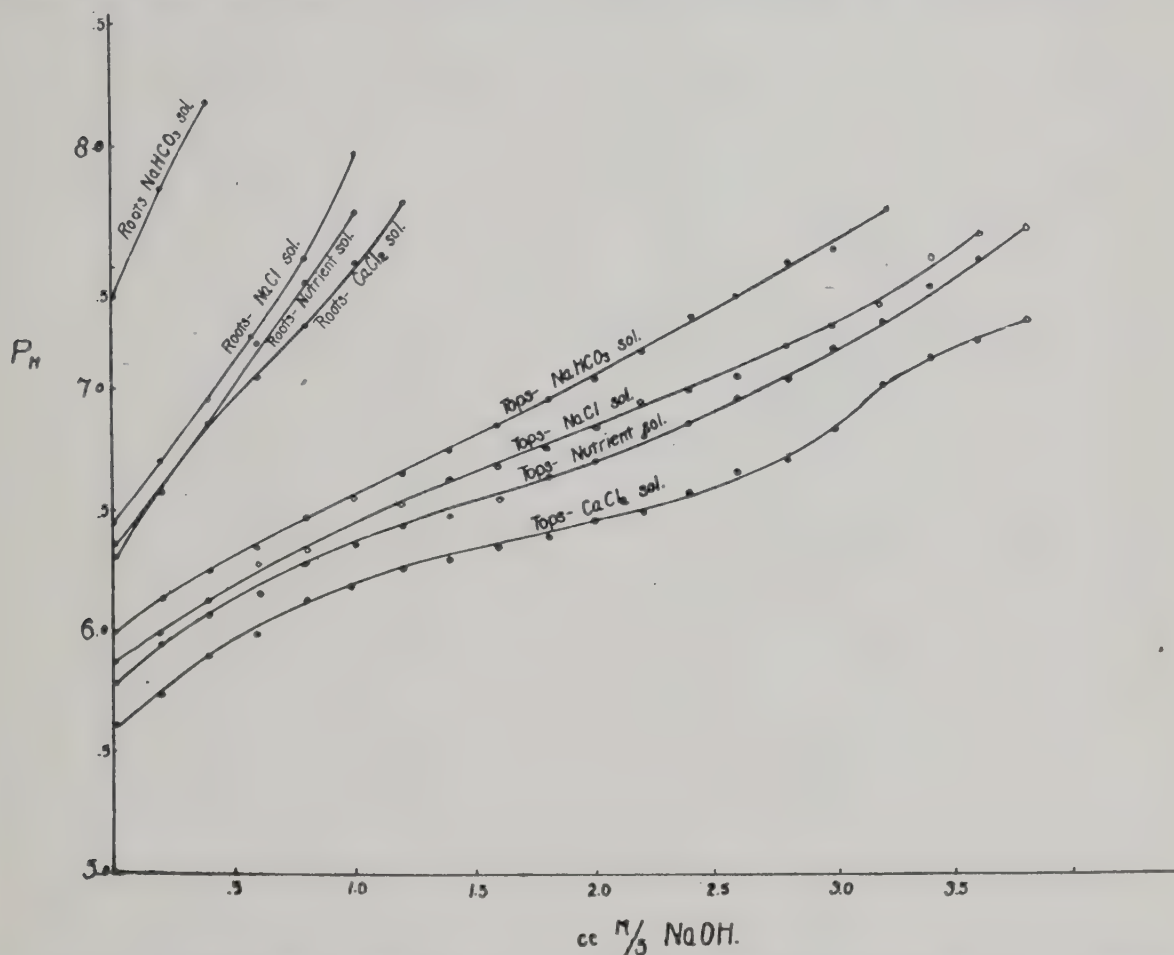


Fig. 3. Titration curves for sap (25 c.c.) expressed from barley plants grown (together with pumpkins) in nutrient solution, and in nutrient solutions with the addition of various salts. For details, see table 6, Experiment C.

Sodium bicarbonate caused the reaction to be changed from acid to alkaline, a change which was accompanied by marked injury to the roots.

Since the concentrations of salts were different in the two experiments just described, another experiment was carried out in which barley and pumpkins were grown in the same solution, each pan containing both kinds of plants, divided half and half. Smaller concentrations of salts were used (table 6). The general relations between the different curves are very similar to those obtained in the previous experiments (figs. 3 and 4).

TABLE 6.—DETAILS OF EXPERIMENTS TO WHICH PLATES 1 TO 5 REFER

Experiment A (Barley)

Salts added to 4 liters of nutrient solution in several portions:			Total R.V.
NaCl	20 g.—10 g.—10 g.....		171
NaHCO <sub>3</sub>	12 g.— 8 g.—10 g.—10 g. ....		120
CaCl <sub>2</sub> .2H <sub>2</sub> O	31 g.—15 g.—15 g. ....		188
NaNO <sub>3</sub>	30 g.—15 g.—15 g. ....		178

Except in solution to which NaHCO<sub>3</sub> was added, the reaction was maintained as approximately neutral. Reaction in nutrient solution + NaHCO<sub>3</sub> pH 8.1—8.8.

Marked injury to whole plant with CaCl<sub>2</sub>; greatest injury to roots with NaHCO<sub>3</sub>; similar but less injury with NaNO<sub>3</sub>; slight injury to tops with NaNO<sub>3</sub>, NaHCO<sub>3</sub>, and NaCl.

All plants grown 17 days in nutrient solution and 19 days in solutions described above. (April 29—May 17.)

Experiment B (Pumpkins)

Salts added to 4 liters of nutrient solution:			Total R.V.
NaCl	10.2 g. ....		44
NaHCO <sub>3</sub>	14.8 g. ....		44
CaCl <sub>2</sub> .2H <sub>2</sub> O	12.5 g. ....		39

Plants grown 2 weeks in nutrient solution and 12 days in solutions described above. (March 7—March 18.)

Some yellowing of plants in NaHCO<sub>3</sub> solution.

Experiment C (Barley and Pumpkins)

Salts added to 4 liters of nutrient solution:			Total R.V.
CaCl <sub>2</sub> .2H <sub>2</sub> O	31 g. ....		95
NaHCO <sub>3</sub>	20 g. ....		60
NaCl	20 g. ....		86

pH values at end of experiment:

Nutrient solution.....	6.8
Nutrient solution plus CaCl <sub>2</sub> .....	6.8
Nutrient solution plus NaCl.....	6.8
Nutrient solution plus NaHCO <sub>3</sub> .....	8.6

Plants grown 3 weeks in nutrient solution and 1 week in solutions described above. (July 13—July 19.)

Marked injury to barley plants with CaCl<sub>2</sub>; slight injury with NaCl; injury to pumpkins in all solutions; greatest with NaHCO<sub>3</sub>. Root injury to both barley and pumpkins with NaHCO<sub>3</sub>.

Experiment D (Peas)

Salts added to 4 liters of nutrient solution:			Total R.V.
NaCl	20 g. ....		86
CaCl <sub>2</sub> .2H <sub>2</sub> O	31 g. ....		95
NaHCO <sub>3</sub>	20 g. ....		60

Plants grown 3 weeks in nutrient solution and 1 week in solutions described above. (September 8–16.)

Appreciable injury with NaHCO<sub>3</sub> and NaCl solutions, less in CaCl<sub>2</sub> solution.



A similar experiment was also made with peas (fig. 5). No appreciable change in hydrogen ion concentration was produced in the sap expressed from the tops, although some displacement of the curves for calcium chlorid and sodium bicarbonate may be noted. The only extensive change in the root sap occurred when sodium bicarbonate was present in the solution.

It may be concluded from these examinations on plant sap that the buffer effect of the roots is in all cases definitely smaller than that

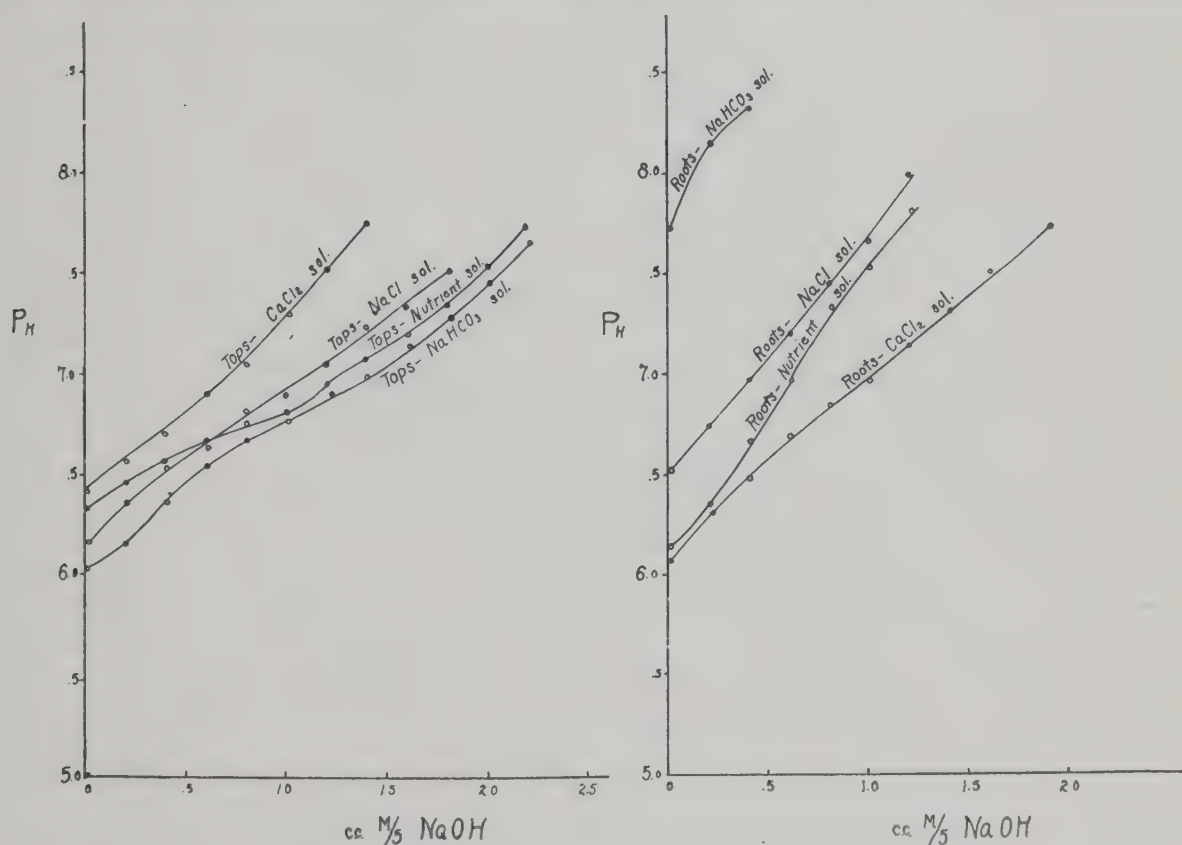


Fig. 4. Titration curves for the sap (25 c.c.) expressed from pumpkin plants grown (together with barley) in nutrient solution, and in nutrient solutions with the addition of various salts. For details, see table 6, Experiment C.

of the tops, a fact which is in agreement with the data obtained by Haas<sup>5</sup> and Kappen.<sup>6</sup> Alkaline solutions, such as are formed even with relatively moderate concentrations of sodium bicarbonate, may produce very marked changes in the reaction of the root sap, while the sap expressed from the tops is more difficult to alter, though with more prolonged treatment its reaction may likewise be changed. Other salts may also affect the reaction and buffer system of the sap, and in this respect calcium chlorid in the higher concentrations seems to exercise a significant influence, also sodium nitrate. It may be added that small increases in the pH value of the expressed sap may cause certain constituents to precipitate.

The determinations of hydrogen ion concentration and titration curves were made by means of a hydrogen electrode as described elsewhere. Such determinations do not have a high degree of accuracy, but can usually be duplicated to within .1 pH. Under the conditions of the experiments, reduction of nitrate did not affect the results, as is shown by the fact that the root sap gave similar values by both colorimetric and electrometric methods. An exception was found in the case of the roots grown in the high sodium nitrate solutions.

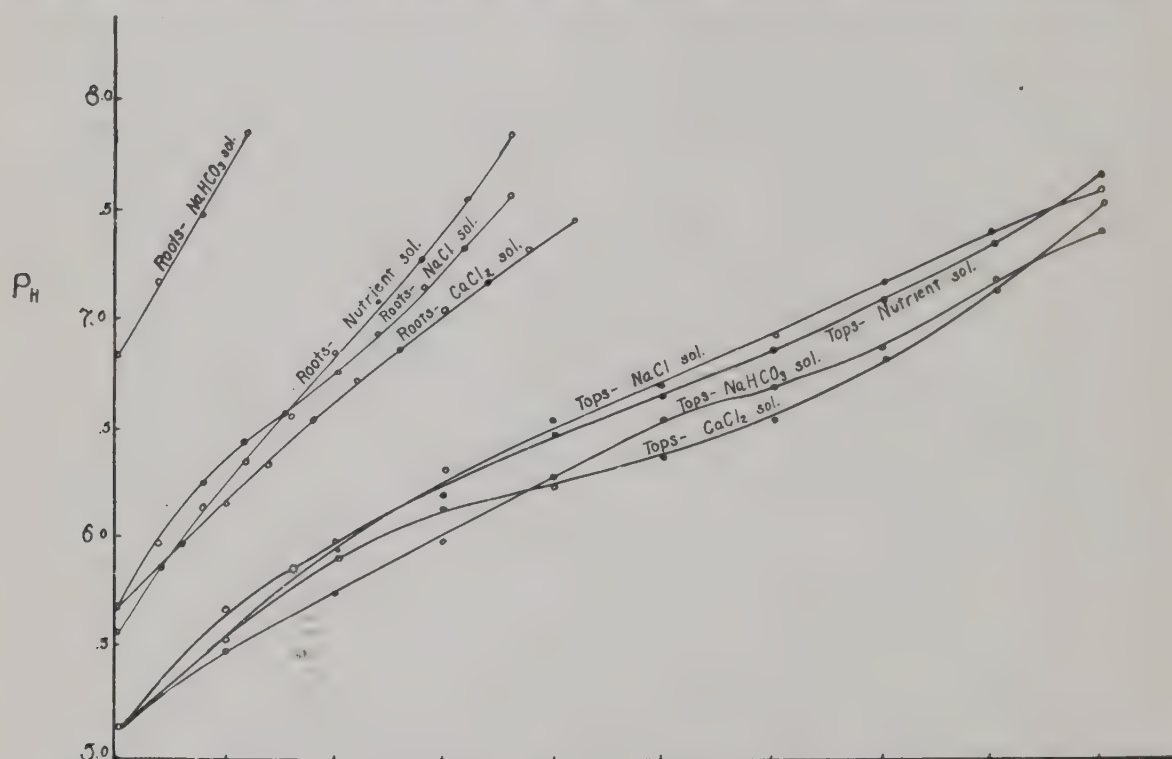


Fig. 5. Titration curves for the sap (25 c.c.) expressed from pea plants grown in nutrient solution, and in nutrient solutions with the addition of various salts. For details, see table 6, Experiment D.

At the time that the reaction of the sap was determined, observations were also made on its osmotic value as shown by freezing point depressions (table 7). It was hoped that these measurements would give some idea of the relations existing between the solution and the sap expressed from roots and tops of plants grown in solutions of various salts and exhibiting different degrees of injury. In all cases the osmotic values of the sap were increased, as was to be expected. The greatest increases were produced by sodium chlorid, which, especially in the roots, has a considerably greater effect than calcium chlorid. In several cases the root sap had a lower osmotic value than the solution in contact with the roots. The different plants attain different equilibrium points in this regard; for example, with the



TABLE 7—FREEZING POINT DEPRESSIONS OF SAP EXPRESSED FROM PLANTS GROWING IN VARIOUS SOLUTIONS

Experiment A			
	Solution at end of experiment °C.	Sap from tops °C.	Sap from roots °C.
Barley, nutrient solution.....	.029	.637	.325
Barley, nutrient solution plus NaCl.....	.592	1.172	.460
Barley, nutrient solution plus CaCl <sub>2</sub> .....	.462	.935	.367
Barley, nutrient solution plus NaHCO <sub>3</sub> .....	.367	1.087	.365
Barley, nutrient solution plus NaNO <sub>3</sub> .....	.612	1.137	.445
Experiment C			
Barley, nutrient solution.....	.007	.545	.263
Pumpkin, nutrient solution.....	.....	.437	.243
Barley, nutrient solution plus NaCl.....	.317	.902	.475
Pumpkin, nutrient solution plus NaCl.....	.....	.655	.435
Barley, nutrient solution plus CaCl <sub>2</sub> .....	.287	.602	.271
Pumpkin, nutrient solution plus CaCl <sub>2</sub> .....	.....	.567	.343
Barley, nutrient solution plus NaHCO <sub>3</sub> .....	.247	.692	.328
Pumpkin, nutrient solution plus NaHCO <sub>3</sub> .....	.....	.557	.393
Experiment D			
Peas, nutrient solution.....	.054	.549	.261
Peas, nutrient solution plus NaCl.....	.439	.884	.391
Peas, nutrient solution plus CaCl <sub>2</sub> .....	.400	.704	.306
Peas, nutrient solution plus NaHCO <sub>3</sub> .....	.286	.567	.376

(For details of experiments see Table 6.)

same solution barley was able to increase the osmotic pressure of the sap to a greater extent than pumpkins could in the given time. Conductivity measurements were also made on the plant juices, and these gave evidence that most of the increases of osmotic pressure may be ascribed to electrolytes.

DISCUSSION

All of the experiments described in this paper lead to the conclusion that the presence of ‘alkali’ salts in the nutrient solution causes marked changes in the absorption of inorganic elements by the plants under consideration. These changes may occur even when the concentration of salt is not sufficiently high to produce great injury. Alterations may likewise be brought about in the reaction and buffer systems of the tops and roots. It is open to question, however, whether

any considerable modifications in the normal reaction of the sap occur except when accompanied by definite injury. (Some recent work on this question is reported by Bauer and Haas.<sup>7</sup>) The roots are especially susceptible to such injury and change of reaction when an alkaline solution of high buffer value is used. Doubtless alterations in permeability occur and the buffer effect in the roots is not sufficiently strong to maintain the normal reaction, even when the contact with sodium bicarbonate solutions is relatively brief. In the case of a culture solution containing calcium chlorid, no unfavorable hydrogen ion concentration is present in the solution. Nevertheless, the reaction and buffer system of the sap may suffer certain modifications. Absorption studies made with barley have shown that the intake of chlorin may be considerably more rapid than that of calcium, equilibrium in the solution being maintained by the excretion or formation of bicarbonate ions. This must be equivalent to the introduction of a strong acid into the plant, with a tendency toward increased hydrogen ion concentration in the sap. But other types of plants may respond in a different manner. Thus the tendency with pumpkins was in the direction of a decrease in acidity of the sap expressed from the tops of the plants. It may be suggested that in this case certain of the organic acids present were precipitated or neutralized by the absorbed calcium. No studies have, as yet been made on the absorption of calcium and chlorin by pumpkins.

Several factors are doubtless concerned in the depression of absorption of certain inorganic ions caused by the presence of sodium salts. A large excess of sodium ions might be expected to bring about a certain displacement of other cations in any chemical compounds involved in the processes of absorption or utilization. Permeability relations may also be modified. With barley, considerable changes of this nature may occur without any evidences of marked injury. It is possible, however, that other plants might be much more susceptible to such modifications in the intake of certain important elements. Also, it may be suggested that under certain soil conditions, a high sodium content in the soil solution may be accompanied by a relatively low concentration of one or more culture elements, for example, calcium. A concentration of calcium, in itself adequate, might conceivably become inadequate when sodium was present in too high concentration. These relations would apparently



partake more of the nature of nutritional effects than of antagonism in the sense in which this term is employed by Osterhout. (See Reed and Haas.<sup>8</sup>) It is true, however, that concentrations in the plant sap are likely to be far higher than those in the culture solution, so that internally saturated surface effects are not out of the question. In certain experiments additional calcium chlorid or calcium sulfate was added to nutrient solutions containing inhibiting concentrations of sodium-chlorid or sulfate, without any resulting increase of growth. The use of solutions containing very low concentrations of calcium would no doubt cause further injury. This relation between sodium salts and deficient nutrient solutions is now being studied.

The detailed discussion of the influence of hydrogen ion concentration on the absorption of culture elements is being considered in another investigation to be reported by Theron.<sup>9</sup> In this connection Breazeale<sup>10</sup> carried out a series of experiments with seedlings to show the influence of sodium salts on the absorption of nitrogen, phosphorus, and potassium from culture solutions. Calcium carbonate was present in excess and the principal effects produced on the absorption of the elements referred to above are attributed to sodium carbonate either formed by inter-reactions between the salts or added originally. Calcium and magnesium were not determined.

If we consider, from a more general point of view, the effects on a plant of a so-called alkali condition in the culture solution, or in the soil solution, we are brought to the conclusion that no simple explanation will suffice. In the first place, many diverse conditions in the soil are referred to under the general term of 'alkali.' Even when we distinguish between alkaline and saline soils, we have defined the nature of the soil solution in only the crudest manner. Data which would enable any accurate classification to be made with regard to the physiological properties of alkali soils are not available at the present time.

One of the most obvious types of injury to plants growing in a saline soil is generally ascribed to unfavorable osmotic relations existing between the plant and the solution. It cannot be questioned that interference with the water intake of a plant may cause injury and even death as a result of high concentrations of electrolytes in the soil solution. We have here, however, as modifying influences the adaptability of the plant and the nature of the atmospheric environ-

ment. From the standpoint of water relations alone, the ability of some plants to absorb and store in their sap large quantities of the alkali salts present in the soil solution may be favorable, rather than otherwise, in that the necessary readjustment of the osmotic gradient may readily take place.

Some of the most perplexing cases of malnutrition are found under conditions making it improbable that osmotic forces are primarily concerned. Thus the presence of sodium salts in the culture medium may influence the general nutrition of the plant in the manner already described. Another environment in the solution highly unfavorable to the growth of most useful plants is generally associated with a high intensity of alkalinity. In solution cultures depression of growth occurs in the case of many plants of agricultural interest (including wheat, barley, peas, alfalfa, melons, etc.) when the pH value of the solution rises much above 8. This effect is produced even when complete culture solutions are employed containing as large a concentration of calcium as can be held in solution at the alkaline reaction. Solutions of similar composition are found to be entirely favorable to growth when the reaction is made slightly acid. That a high concentration of calcium may tend, with certain plants, to prevent injury otherwise caused by excessive hydroxyl ion concentration is suggested by an interesting experiment carried out by Reed and Haas<sup>11</sup> in which walnut seedlings were grown for a week or more in continuously renewed solutions of calcium hydrate. Following out this idea, a similar experiment was carried on in this laboratory with wheat, but the plants succumbed after a very short period. It is undoubtedly true that the roots of certain plants are able to make growth in solutions of hydroxyl ion concentration entirely prohibitive of development in the case of most agricultural plants. For example, Bermuda grass will grow fairly well in highly alkaline solutions (not so well, however, as in slightly acid solutions). One reason for its ability to grow under such conditions may be related to the nature of the root structure. A microscopic examination of Bermuda grass roots shows that both the type of cell and the cell arrangement of this grass are very different from those of plants easily injured by the alkalinity of a solution. We further suggest that the roots of different plants may vary widely in their organic composition and that this fact may have some bearing on degrees of tolerance to high alkalinity. It is hoped



that investigations now being conducted will make it possible to reach more definite conclusions on this point.

When the alkalinity of the culture or soil solution is sufficiently intense, rapid disintegration of the roots of the plant grown in it is ordinarily observed. The chemical composition of root tissue is but slightly understood, but it may be suggested that pectin bodies and perhaps proteins and lipoids would be particularly subject to change in an alkaline medium. With moderate alkalinity, the injury to the plant may sometimes not become manifest until after one or two months of growth. With some plants a marked chlorosis occurs. This may, or may not, be the direct result of too high a concentration of hydroxyl ion. Nutritive disturbances must also be considered, and it is possible that the absorption or assimilation of iron or other elements is involved. Direct experiments by Theron<sup>9</sup> show that the reaction of the solution modifies the relative absorption of the ions present, the absorption of nitrate being decreased in an alkaline medium.

Haas<sup>12</sup> Hempel,<sup>13</sup> and Kappen<sup>14</sup> have carried on interesting and important investigations on the buffer system of plants, but this subject has not yet received the same detailed study which has been devoted to the buffer system of the blood. It is very probable, however, that the maintenance of a proper reaction in the living plant cell is also of great importance. Unfortunately, the juices which are expressed from the plant tissue do not represent anything so definite as the blood and do not normally display such constancy of reaction, yet it is possible that any considerable change in reaction or buffer effect of the expressed sap induced by alkali salts implies some important disturbance of metabolism and colloidal condition of the protoplasm. The evidence presented in this paper suggests that such changes of reaction may be produced in plants by certain salts, particularly in the roots. Appreciable quantities of hydrolyzable salts, such as sodium bicarbonate, may be very effective in modifying the reaction of the sap, even though the solution may not show an extremely high pH value. The reserve of hydrolyzable salt and the continued maintenance of an alkaline reaction, as well as the pH value at any given moment, are important from a physiological standpoint. Also, as stated before, a solution of approximately neutral reaction may cause some modification of reaction in the plant sap,

as is shown by the results obtained with solutions containing non-hydrolyzable sodium salts or calcium chlorid. Excess of sodium nitrate acts to a certain extent like sodium bicarbonate. This fact is explainable by the rapid utilization of the nitrate ion with the formation of an alkaline residue.

Whatever may be the exact significance of the differences of reaction, it is evident that the internal chemical system of a plant may be greatly modified under so-called alkali conditions as a result of changes induced in the relations between the absorbed ions and organic complexes synthesized by the plant.

In soils maintaining an alkaline reaction, certain elements, such as calcium, magnesium, and iron may occur in the soil solution only in very low concentrations. It may be inferred that in such a medium, most plants might find it impossible to absorb certain essential elements at a sufficiently rapid rate to permit of satisfactory growth. The plant itself, however, tends to overcome the unfavorable reaction and deficiency of solutes by its ability to excrete carbon dioxide. Whether or not the desired plant growth takes place, will depend upon the buffer effect of the soil, the nature and rate of solution of the mineral components, and of course upon physical conditions, which are not now under consideration. Specific toxic compounds, organic or inorganic, other than those discussed have been suggested as contributing causes of injury, but no extensive data dealing with this phase of the question are available.

Any discussion of the effects of salts on plants would be incomplete without reference to the part played by climatic influences. The same solution may vary in toxicity to a very great extent according to the nature of the aerial environment, temperature, sunlight, and humidity. Absorption and transpiration of water and the intake of the ions of alkali salts and of the nutrient elements, may all undergo significant modification when climatic conditions are varied. It is not to be expected, therefore, that any sharp line of demarcation can be drawn between toxic and non-toxic solutions, any more than between good and poor nutrient solutions. Lipman and Davis<sup>15</sup> have shown, experimentally, that various concentrations of sodium chlorid may stimulate growth under certain conditions, while other experiments, conducted at a different time of year, gave evidence that similar solutions produced marked inhibition of growth under the new environment.



## SUMMARY

1. Sodium chlorid and sodium sulfate, when added to a culture solution, caused certain marked alterations in the absorption of inorganic elements and in the composition of the barley plant. The cations were particularly involved, the sodium salts tending to decrease the absorption of calcium, magnesium, and potassium.

2. When sodium chlorid is used, sodium and chlorin may be absorbed and stored by the barley plant in relatively large quantities. In the case of sodium sulfate, the sulfate ion is removed from solution less rapidly than the chlorin ion. This is also true of other plants than barley, experiments with cucumbers and cantaloupes having given similar results.

3. On the basis of preliminary experiments, the question is raised whether sodium chlorid possesses greater toxicity than sodium sulfate for common agricultural plants, when equal osmotic values, or equal concentrations of sodium are compared. It is concluded that no definite alkali tolerances for different plants can be established, because of the very important modifying influences of climate and season and other environmental conditions.

4. Observations were made on the effect of salts on the reaction and buffer systems of barley, peas, and pumpkins. Rapid and extreme changes in the reaction of the sap expressed from the roots were caused by the addition of sodium bicarbonate to the culture solution. The buffer effect of the sap expressed from the stems and leaves was greater than that of the sap expressed from the roots, and less subject to change of reaction. Calcium chlorid produced appreciable changes in the reaction and buffer effect of the plant juices. Barley and pumpkins were influenced in opposite directions. Neutral sodium salts also caused slight changes in reaction and titration values. Sodium nitrate in the concentration employed increased the alkalinity of the expressed root sap, with accompanying injury similar to, although less extensive than that induced by sodium bicarbonate.

5. A brief general discussion of certain phases of alkali injury to plants is given.

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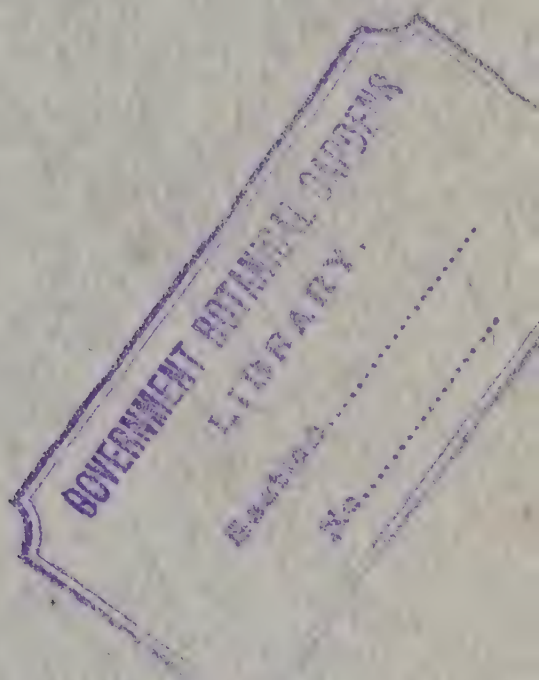
AUGUST, 1923

TECHNICAL PAPER No. 9

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EXPERIMENTS ON THE RECLAMATION OF  
ALKALI SOILS BY LEACHING WITH  
WATER AND GYPSUM

BY  
P. L. HIBBARD



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EXPERIMENTS ON THE RECLAMATION OF  
ALKALI SOILS BY LEACHING WITH  
WATER AND GYPSUM

BY

P. L. HIBBARD

(Contribution from the Division of Plant Nutrition, California Agricultural Experiment Station)

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In connection with an investigation of the effects of drainage and flooding on certain alkali soils found on the Kearney Vineyard Ranch of the University of California, one-ton lots of five different types of these soils were brought to the laboratories at Berkeley and have been given intensive study for a period of several years. The results of various pot experiments on these soils have already been reported elsewhere.<sup>1</sup> These observations indicated that it would be necessary to remove most of the alkali before successful crops could be grown. The present discussion is especially concerned with the detailed chemical examination of columns of soil which had been subjected to leaching and to gypsum treatments. In view of the importance of the alkali problems which are suggested by the study of these soils, and because of the intensive experiments in the field now being conducted by Kelley and Thomas, it seems desirable to place on record certain of the data obtained in this laboratory.

Complete analyses and descriptions of the soils were given in the article mentioned.<sup>1</sup> In general, they are classified as Madera fine sandy loam. Numbers 16, 17, 19, and 20 are very similar physically. No. 18 is of finer texture and contains more clay.

Chemically, these soils vary chiefly in their content of easily soluble salts.

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<sup>1</sup> Soil Science, vol. 13, p. 125 (Feb., 1922).

No. 16 is so highly alkaline and saline that no vegetation grows upon it.

No. 17 is similar, but has been leached in the field by flooding so that it now contains less than one-fourth as much soluble matter as 16. It also is without vegetation.

No. 18 is neutral in reaction, but so high in salt content that little will grow on it.

No. 19 contains moderate amounts of both alkaline and neutral salts. Alkali-tolerant crops grow on this soil.

No. 20 has much more alkaline and neutral salts than 19, but less than 17. Few plants grow well in it.

Table 1 shows the total amount of water-soluble alkaline and neutral salts in these soils; and the column of table 2 headed "Before Leaching," shows the amount of each of the principal ions in the original soils.

TABLE 1  
CHANGES IN ALKALI AND SALTS BY FIRST LEACHING

<i>Soil No.:</i>	16	17	18	19	20
Weight of original soil, in pounds.....	105	102	97	102	104
Total depth of water added; in feet.....	1.5	1.5	1.5	1.5	1.5
Time after adding water before first drip; in days.....	7	1	4	3	2
Total time of leaching; in days.....	80	72	63	68	67
Approximate per cent of alkalinity as $\text{Na}_2\text{CO}_3$ at start.....	1.06	0.32	0	0.09	0.16
Approximate per cent of alkalinity as $\text{Na}_2\text{CO}_3$ at end.....	0.42	0.13	0	0.03	0.08
Approximate per cent of salts at start.....	1.61	0.30	0.82	0.14	0.33
Approximate per cent of salts at end.....	0.20	0.05	0.16	0.04	0.03

Experiments described elsewhere (see note 2) showed that addition of gypsum and other amendments did not overcome the toxicity of these soils sufficiently to permit the production of satisfactory crops. It was therefore decided to see what could be accomplished by leaching. The new experiments were designed to determine quantitatively, as nearly as possible on a laboratory scale, the changes taking place in these soils when leached with water containing gypsum in solution. The chief points studied were:

1. Rate of leaching, or time required to free the soil of excessive alkaline and neutral salts.



2. Amount of water necessary to accomplish this leaching.
3. Quantity of soluble matter removed.
4. Depth of soil from which this soluble matter was removed by a limited amount of water.

TABLE 2

QUANTITY AND LOCATION OF THE CHIEF WATER-SOLUBLE ANIONS IN THE SOILS  
BEFORE AND AFTER THE FIRST LEACHING

Parts per million in dry soil

Soil 16					
	Before leaching	After leaching			
		Top	1 ft.	2 ft.	3 ft.
pH.....	9+	8	9+	9+	9+
Ca.....	0	300	0	0	0
CO <sub>3</sub> .....	3300	72	240	648	888
HCO <sub>3</sub> .....	3050	342	488	512	634
SO <sub>4</sub> .....	2000	2000	1000	1000	1000
Cl.....	7600	100	100	140	200
Soil 17					
pH.....	9+	8	9	9+	9+
Ca.....	0	100	0	0	0
CO <sub>3</sub> .....	600	48	168	528	720
HCO <sub>3</sub> .....	610	342	512	512	488
SO <sub>4</sub> .....	200	400	1000	1000	1000
Cl.....	1850	80	100	120	160
Soil 18					
pH.....	7	7.5	8	8	8
Ca.....	300	0	0	0	0
CO <sub>3</sub> .....	0	0	48	72	48
HCO <sub>3</sub> .....	61	340	560	537	340
SO <sub>4</sub> .....	1250	0	0	0	0
Cl.....	1275	0	0	100	260
Soil 19					
pH.....	8.2	7.2	8	8.5	9
Ca.....	0	0	0	0	0
CO <sub>3</sub> .....	48	48	96	192	240
HCO <sub>3</sub> .....	238	410	537	586	732
SO <sub>4</sub> .....	500	0	0	0	0
Cl.....	635	0	0	60	140
Soil 20					
pH.....	8.5	8	8.5	9	9
Ca.....	0	0	0	0	0
CO <sub>3</sub> .....	120	48	120	240	384
HCO <sub>3</sub> .....	183	390	439	439	268
SO <sub>4</sub> .....	500	0	0	0	0
Cl.....	1825	0	0	0	0

5. Possibility of return of the remaining soluble matter toward the surface when it had not all been removed from the lower layers of the soils.

6. Suitability of the leached soil for the growth of crops.

The soils were placed in iron sewer pipes about 6 inches in diameter and 5 feet long, tarred inside and outside. These pipes were supported in a vertical position with the bell end up, the lower end hanging free. To retain the soil, the lower end was covered with muslin supported by a strong wire gauze tied to the end of the pipe. In filling the pipes, a small amount of the dry soil was moistened and packed into the bottom of the pipe. Then the dry soil, together with some water, was added, with stirring and packing, until the pipe was full. This procedure was followed in order to avoid the considerable contraction in volume which the soil would otherwise have suffered when covered with water. In this way the pipes remained nearly full after the leaching was completed.

Gypsum was added to only the top foot of the soil, in the amount calculated to be necessary for changing the sodium carbonate in this portion of the soil to sodium sulfate. After filling with soil, the small space at the top of the pipes was kept covered, 1 to 2 inches deep, with water containing  $\frac{1}{10}$  per cent of gypsum until 8250 c.c. of water had been added to each. This quantity is equivalent to a depth of  $1\frac{1}{2}$  feet of water, probably more than would ordinarily be applied at one time in field flooding.

Percolation was comparatively rapid at first, until most of the salts had been removed, after which the rate decreased gradually. After two months, percolation became very slow. The most rapid flow was always through soil 18. This soil is finer in texture and contains more clay than any of the others; but it contains only neutral salts and no  $\text{Na}_2\text{CO}_3$ , so that it was less deflocculated by the leaching than were the others. The remaining four soils were physically similar, and percolation through them was approximately inversely proportional to their content of  $\text{Na}_2\text{CO}_3$ . Soil 16 became very nearly impervious after two months' leaching. In table 1 are summarized some of the data in respect to time and effectiveness of leaching, and the approximate alkalinity and salinity of the soils before and after leaching with  $1\frac{1}{2}$  feet of water, which is less than one-third of the



volume of the soil treated. This water removed from 70 to 90 per cent of the neutral salts in the soil, but only one-fourth to one-half of the total alkaline salts.

#### LOCATION OF THE REMAINING ALKALI AFTER THE FIRST LEACHING

After the soils had dried out sufficiently, analyses were made of samples taken from the top and from the first, the second, and the third foot. The results are given in table 2. The composition of the unleached soil is given in the first column. The analytical methods used were only approximate, but all samples were tested by the same methods, so that the results are comparable. When  $\text{SO}_4$  and Ca appear in the top soil, it is assumed that they have been derived from unchanged gypsum which was added to the top foot of soil before leaching. This is most apparent in soils 16 and 17, to which large amounts of gypsum were added.

It appears that, in every case, nearly all the chlorid was removed to below the three-foot level in the soil. Most of the  $\text{SO}_4$  also was washed down below three feet. Carbonate ( $\text{CO}_3$ ) was nearly all removed from the top, but much remained in the lower portions of the soil. Soil 18 had apparently gained in concentration of hydroxyl ions by the leaching. This effect is commonly produced by leaching the sodium salts from a neutral soil.<sup>2</sup> In the third foot of soil 18, the carbonate and bicarbonate are less than in the second foot, but here a considerable portion of the original neutral salts remains and depresses the alkalinity. It is well known that the addition of neutral salts to a slightly alkaline solution lowers the pH of the solution.<sup>3</sup>

Seemingly, the top soil in each column had been sufficiently cleared of alkali by the leaching to permit the growth of a crop. Accordingly, barley was planted in each on July 15, while the soil was still very wet. While the number of plants grown was too small to justify other than very general conclusions, it was apparent that while the soils were no longer toxic, they were, nevertheless, incapable of producing normal plants, probably because of a lack of available nutrients. In another series of experiments, it was found that soil 18 *after leaching* produced nearly as good a crop of barley as an otherwise similar soil which had never contained a high concentration of salts.

<sup>2</sup> Cf. Cummins and Kelley, Univ. Calif. Agri. Exp. Sta. Tech, Paper No. 3.

<sup>3</sup> See Clarke, *Determination of Hydrogen Ions*, p. 84.

## RISE OF ALKALI DURING GROWTH OF BARLEY

One of the chief points to be determined in this investigation was whether there would be a sufficient return of alkali to the surface, from the lower part of the soil, to produce a toxic concentration in the zone of plant roots. To give the greatest possible opportunity for a rise of the alkali, the lower ends of the soil columns were kept in jars of distilled water. After the plants were well started, no water was applied to the surface of the soil; consequently the plants had to draw their moisture from the lower part of the columns which still contained alkali. On account of the small growth of the barley, there was no great loss of water by transpiration. There was considerable evaporation from the surface of the soil, however, so that there was some rise of salts. After removal of the crop, the soils were again sampled and analyzed in the same manner as after the first leaching. It was so difficult to obtain the samples that it cannot be assumed that they were taken from exactly the same locality in the soil in both cases. They show fairly well, however, what changes had taken place during the growth of the crop. The results are given in table 3.

It appears that alkalinity, as measured by  $\text{CO}_3$  and  $\text{HCO}_3$ , had decreased in nearly all cases. But salinity, as measured by  $\text{Cl}$ , had returned toward the tops of the soil columns. Though the amount of variation is not very great, it is enough to show the tendency of the salts to rise with the capillary soil moisture. Before it can be said that an alkali soil has been reclaimed by flooding and drainage, it is evident that soluble matter will have to be removed by leaching to such a depth that the ordinary movements of soil moisture will not again bring it up to the region of plant roots.

To explain the lessened alkalinity after the growth of the first crop, the following hypothesis is offered: At the time of planting the newly leached soils were full of water and consequently contained very little air, but there was considerable gypsum in the upper foot. During the growth of the crop, the soils were well aerated, and some  $\text{CO}_2$  was released by the plant roots. Also, the free oxygen helped to form more  $\text{CO}_2$  by oxidizing organic matter in the soil. The increased  $\text{CO}_2$  changed  $\text{Na}_2\text{CO}_3$  to  $\text{NaHCO}_3$ , and the  $\text{CaSO}_4$  changed  $\text{NaHCO}_3$  to  $\text{Na}_2\text{SO}_4$ , so that the alkalinity was reduced.



THE SECOND LEACHING

After the crop of barley was harvested, leaching was again started in order to remove the residual soluble salts in the lower parts of the soil columns. The soils were kept covered most of the time with water saturated with gypsum. This treatment was continued from Novem-

TABLE 3  
QUANTITY AND LOCATION OF THE CHIEF WATER-SOLUBLE ANIONS IN THE SOILS  
AFTER GROWTH OF FIRST CROP  
Parts per million in dry soil

	Soil 16			
	Top	1 ft.	2 ft.	3 ft.
pH.....	8	8.5	9.0	9.5
Ca.....	1200	50	25	0
CO <sub>3</sub> .....	0	30	360	480
HCO <sub>3</sub> .....	183	427	670	427
SO <sub>4</sub> .....	1700	250	0	0
Cl.....	30	30	175	200
	Soil 17			
pH.....	8	8.5	9.0	9.5
Ca.....	300	100	25	50
CO <sub>3</sub> .....	0	60	240	360
HCO <sub>3</sub> .....	150	458	610	793
SO <sub>4</sub> .....	600	0	100	0
Cl.....	20	25	225	350
	Soil 18			
pH.....	7.5	8.0	8.0	7.5
Ca.....	50	25	25	150
CO <sub>3</sub> .....	0	0	0	0
HCO <sub>3</sub> .....	122	213	183	61
SO <sub>4</sub> .....	0	100	1000	250
Cl.....	20	50	390	585
	Soil 19			
pH.....	8.5	8.5	8.5	8.5
Ca.....	100	0	0	0
CO <sub>3</sub> .....	0	30	42	48
HCO <sub>3</sub> .....	183	305	341	390
SO <sub>4</sub> .....	1000	500	500	500
Cl.....	110	225	335	350
	Soil 20			
pH.....	8.0	8.5	9.0	9.0
Ca.....	100	0	0	0
CO <sub>3</sub> .....	30	120	180	180
HCO <sub>3</sub> .....	275	305	366	335
SO <sub>4</sub> .....	1500	0	250	500
Cl.....	25	30	75	250

ber 1, 1920, to May, 1921. Percolation was slow through all of them except soil 18, which was soon nearly free of saline matter. The rate of flow was very slow through soils 16 and 17, which were still very alkaline. On May 4, the percolates were tested and found nearly free of  $\text{CO}_3$ ,  $\text{SO}_4$ , and  $\text{Cl}$ , except that from soil 16, which still contained some  $\text{CO}_3$ ,  $\text{HCO}_3$ , and  $\text{Cl}$ . At this time, about a three-inch depth of the solution of gypsum was applied to each. This solution disappeared from the surfaces of soils 18, 19, and 20 in a few days, from soil 17 in 12 days, and from soil 16 in 20 days. The final percolates, except from soil 16, contained very little soluble matter other than gypsum, which was plentiful in all but those from soil 16 and soil 17. These percolates contained no calcium, but much  $\text{SO}_4$  and a little  $\text{CO}_3$ ,  $\text{HCO}_3$ , and  $\text{Cl}$ . It was considered that soils 18, 19, and 20 had been cleared of easily soluble sodium salts, while some of these still remained in 17, and considerable remained in 16.

Further attempts were then made to grow plants in the leached soils. Barley, cucumbers (apparently very sensitive to salinity and alkalinity), and peas were grown. The same conclusion was reached as before, namely that toxic concentrations of salts were probably absent, but that lack of essential plant nutrients prevented satisfactory plant growth. In following out this idea, complete analyses of water extracts of the soils were made. The results are given in table 4. The extracts were made with carbon dioxide-free-water, in a ratio of 5 parts of water to 1 of soil, and were filtered through Pasteur-Chamberland filters. The soil for these analyses was taken from the top foot only of the soil columns.

These analyses indicate that there is not enough salinity or alkalinity in the top foot of the soil to be injurious to any ordinary plant. Considerable  $\text{CaSO}_4$  is present, but probably not in injurious amounts. Potassium and phosphate are not abundant, but probably are present in quantities sufficient for much more growth than was obtained. The amount of nitrate is very low, and the total amount of nitrogen in the soils is also very low. These deficiencies are thought to partly account for the poor growth.



TIME REQUIRED, GYPSUM USED, AND EFFECTIVENESS OF LEACHING

Table 1 summarized these points for the first leaching. The results as a whole will now be considered.

*Time.*—The first leaching lasted for 63 to 80 days, the second, 6 months. During these periods, the soils were kept covered with water. The removal of salts from soil 18 was completed in considerably less time, but some neutral salts and considerable alkalinity

TABLE 4

ANALYSIS OF WATER EXTRACTS OF LEACHED ALKALI SOILS, DECEMBER, 1921

Parts per million in dry soil

Soil No.:	16	17	18	19	20
Total solids.....	2750	1925	1025	975	1015
Loss on ignition.....	275	225	100	125	165
Soluble SiO <sub>2</sub> .....	68	50	82	50	50
Fe.....	0.75	0.25	0.25	1.50	0.60
Ca.....	618	342	172	207	190
Mg.....	22	22	22	18	16
Na.....	103	121	46	60	54
K.....	21	66	43	42	56
CO <sub>3</sub> .....	0	0	0	0	0
HCO <sub>3</sub> .....	153	92	140	140	140
SO <sub>4</sub> .....	1156	1075	458	515	473
Cl.....	30	15	10	15	10
NO <sub>3</sub> .....	2	8	1	8	2
PO <sub>4</sub> .....	5.50	3.70	8.70	7.50	5.20
Total N per cent.....	0.020	0.019	0.052	0.031	0.023

still remained in soil 16, and a little in soil 17. It may be inferred that complete removal of large amounts of soluble matter from soil in the field would require many months of leaching.<sup>4</sup> Observations not here recorded indicate that the time required may be lessened by allowing the soil to dry out and to aerate after a period of leaching. This increases the rate of percolation when leaching is resumed. Emphasis should also be laid on the fact that the rate of percolation in the field may be far more rapid than in columns of soil, such as those employed in this investigation.

<sup>4</sup> See article by Cameron and Patten, Jour. Am. Chem. Soc., vol. 28, p. 1639 (1906).

TABLE 5  
GYPSUM AND WATER USED IN LEACHING THE SOILS

Soil No.:	16	17	18	19	20
Dry gypsum added before leaching; in grams.....	141	44	4.42	13.1	22.5
Gypsum added in the leaching water; in grams.....	46.69	46.69	78.15	46.69	50.19
Total gypsum added; in grams.....	187.69	90.69	82.57	59.79	72.69
Total gypsum added; in pounds.....	0.231	0.112	0.102	0.074	0.089
Equivalent to tons of gypsum per acre.....	42.5	20.5	19.0	13.5	16.5
Total water used in leaching; in liters.....	32.75	32.75	45.25	31.75	34.75
Equivalent to feet in depth.....	6	6	7.7	5.8	6.3

*Gypsum Used.*—In table 5 are recorded the amounts of gypsum and of water used at various times. With the exception of soil 16 the greater part of the gypsum was added in solution. Soil 16 contained nearly 1 per cent of sodium carbonate, to neutralize which an application of gypsum equal to 32 tons an acre in the top foot was made at the start. The analyses of the leached soils given in table 4 indicate that much of the dry gypsum added at the beginning was never brought into solution. The first leaching was made with water less than half saturated with gypsum, so that it probably dissolved some gypsum from that in the soil. This was probably not the case in the second leaching, since the water in that instance was saturated with gypsum. Very large amounts of gypsum were used, amounts which would not be economically practical in the field. Much of this excess was used to increase the rate of percolation. In field practice, probably much less would be necessary. Nevertheless, as is shown in table 4, all these soils still contain considerable water-soluble sodium, presumably more or less replaceable by calcium. During the leaching the percolates were tested from time to time to find out when the sodium salts were all removed. At the end of the last leaching, the percolate from soil 18 contained much calcium; that from soil 19, a little calcium; that from soil 20, a trace; while in the percolates from soils 16 and 17 no calcium was found. These two soils were still exchanging sodium for the calcium of the gypsum. The upper part of the soil columns contained an excess of gypsum, but the amount of water passing through was not sufficient to carry



the gypsum into the lower layers where it could react with  $\text{Na}_2\text{CO}_3$  so the lower part of the soil was still alkaline. It seems scarcely necessary to point out that gypsum, present in the soil but not dissolved in the soil water, is of no use in overcoming alkalinity. It is less obvious that the deflocculation associated with the alkalinity of such soils decreases percolation so greatly that the real problem is to get enough water through the soil for the solution of an amount of gypsum sufficient to react with all the sodium carbonate. This appears to be one of the chief reasons why so much time is required to remove the alkali from the soils. For example, soil 16 received 32,750 c.c. of water, which would dissolve about 72 grams of gypsum. The calculated amount of gypsum necessary to react with all the  $\text{Na}_2\text{CO}_3$  in this soil is 705 grams. Little more than 10 per cent of this amount could be dissolved in the water applied.

TABLE 6  
PER CENT OF VARIOUS IONS REMOVED BY LEACHING, STATED AS PER CENT OF THE AMOUNT ORIGINALLY PRESENT, AS DETERMINED IN A 1:5 WATER EXTRACT

Soil No.:	16	17	18	19	20
Sodium (Na).....	98	114	98	123	117
Carbonate ( $\text{CO}_3$ ).....	85	67	0	60	50
Bicarbonate ( $\text{HCO}_3$ ).....	61	70	138	59	126
Sulfate ( $\text{SO}_4$ ).....	177	183	190	181	211
Chlorid (Cl).....	87	93	79	103	102
Total solids.....	96	114	88	116	118

*Effectiveness of Leaching.*—In table 6 are given the data showing the per cent of various ions originally present which was removed by the leaching. There is much difference between the percentages of the various ions removed. Carbonate ( $\text{CO}_3$ ) and bicarbonate ( $\text{HCO}_3$ ) were least thoroughly removed; chlorid, most completely. More than 100 per cent of sulfate ( $\text{SO}_4$ ) originally present in the soil was found in the percolates, but much of this was derived from the added gypsum. Very little of the water-soluble sodium originally present remained in the soils. Some soils lost over 100 per cent of the original water-soluble sodium. This is thought to indicate that there was considerable exchange of calcium for sodium in the relatively insoluble minerals of the soil. This change may be regarded as a distinct improvement in the soils. In general, these figures accord very well with the analyses of the final percolates obtained from the soils.

The last percolates, with the exception of those from soil 18, contained considerable carbonate and chlorid, and much bicarbonate, showing that, although the tops of the columns of soils were freed of salts and alkalinity, some still remained in the lower portions.

TABLE 7  
CHANGES IN WATER-SOLUBLE CALCIUM AND SULFATE PRODUCED BY LEACHING  
ALKALI SOILS WITH GYPSUM

<i>Calcium (Ca)</i>	<i>Soil No.:</i>	<i>16</i>	<i>17</i>	<i>18</i>	<i>19</i>	<i>20</i>
Added as gypsum; in lbs....		0.0954	0.0448	0.0419	0.0304	0.0366
Found in percolate; in lbs..		0.0002	0.0005	0.0131	0.0002	0.0005
Net increase in soil; in lbs.		0.0952	0.0443	0.0288	0.0302	0.0361
Net increase in soil; in per cent.....		0.0910	0.0440	0.0290	0.0300	0.0360
<i>Sulfate (SO<sub>4</sub>)</i>						
In original soil; in lbs.....		0.1880	0.0840	0.0660	0.0520	0.0570
Added in gypsum; in lbs. ..		0.2310	0.1120	0.1020	0.0740	0.0890
Total; in lbs.....		0.4190	0.1960	0.1680	0.1260	0.1460
Found in percolate; in lbs.		0.3330	0.1540	0.1240	0.0940	0.1200
Remaining in soil; in lbs.....		0.0860	0.0420	0.0440	0.0320	0.0260
Equal to per cent of SO <sub>4</sub> of original, total.....		46	50	66	61	48
Remaining SO <sub>4</sub> ; per cent in soil.....		0.0820	0.0420	0.0460	0.0320	0.0260
<i>Sodium (Na)</i>						
Per cent in original soil.....		0.9680	0.2050	0.1550	0.0710	0.1670
Per cent of original sodium which has been replaced by calcium.....		9	22	29	40	19

CHANGES IN THE CALCIUM AND SULFATE OF THE SOILS, PRODUCED BY  
LEACHING WITH GYPSUM

Table 7 shows that about half or more of the SO<sub>4</sub> in the original soil has been removed by the leaching. The remaining SO<sub>4</sub> is probably mostly present as CaSO<sub>4</sub>. Since less calcium was found in the percolates than was added as gypsum, it is inferred that the differences may be ascribed to the replacement of sodium with the formation of calcium carbonate or silicates. The sodium thus replaced by calcium would amount to from 9 to 40 per cent of the total originally present in the soils. In soil 16, which had the most sodium, only 9 per cent was replaced by calcium, although 98 per cent of the total water-soluble sodium originally present had been removed. Soil 19,



which had the lowest concentration at first, has had 40 per cent of its sodium replaced by calcium. These statements again make evident the reason why it is so difficult to leach a soil which contains much sodium carbonate, namely, that it is impossible to get a sufficient concentration of gypsum to any point in the soil to neutralize all of the sodium carbonate at that point. The alkalinity must be gradually removed, partly by leaching, and later partly by reaction with gypsum. An electrolyte of much greater solubility, such as  $\text{MgSO}_4$ , or  $\text{CaCl}_2$ , speeds up the percolation greatly.

### CONCLUSIONS

The following conclusions were reached from observations made on the leaching of five-foot columns of five different alkali soils from Kearney Vineyard:

1. Removal of all but negligible amounts of alkaline salts from the first six feet or more of a heavily impregnated soil of the type discussed in this article will require many months of leaching.

2. Soluble matter not carried below the six-foot level by leaching will return toward the surface with the capillary water when the capillary water moves upward.

3. Most of the soluble matter may be leached out by water alone. But gypsum is valuable as a flocculent to increase the rate of leaching.

4. The anions were leached out in the following order: chlorid, nitrate, sulfate, carbonate, bicarbonate.

5. Leaching removes desirable plant food as well as undesirable salts, so that a soil which has been leached long is liable to be very unproductive for some years, or until available nutrients have been accumulated again by suitable agricultural practice.

6. Removal of more than one-half of one per cent of  $\text{Na}_2\text{CO}_3$  from a soil is very slow, because of the high degree of deflocculation produced by the alkalinity and because such a concentration of alkaline salts is much greater than can be neutralized by the gypsum in an equal volume of a saturated solution of gypsum.

7. Rates of percolation through soils may be much more rapid in the field than in constricted columns of soil. Cognizance of this difference must be taken in the application of results of laboratory experiments.

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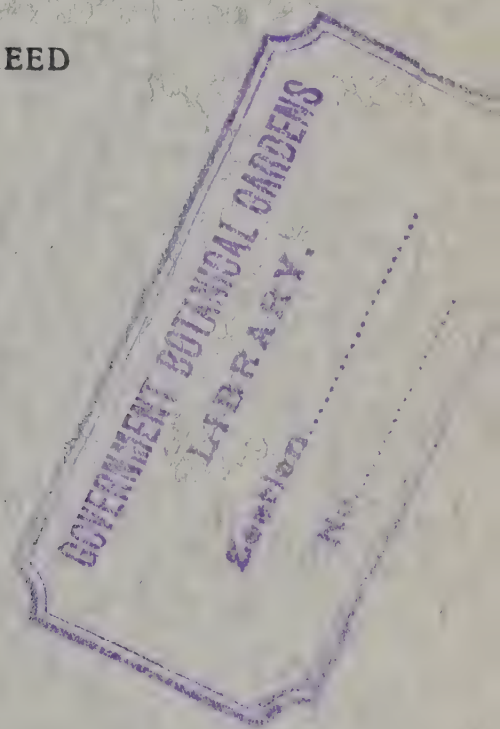
SEPTEMBER, 1923

TECHNICAL PAPER No. 10

THE SEASONAL VARIATION OF THE SOIL MOIS-  
TURE IN A WALNUT GROVE IN RELATION  
TO THE HYGROSCOPIC COEFFICIENT

BY

L. D. BATCHELOR AND H. S. REED



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THE SEASONAL VARIATION OF THE SOIL  
MOISTURE IN A WALNUT GROVE IN  
RELATION TO THE HYGROSCOPIC  
COEFFICIENT\*

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L. D. BATCHELOR AND H. S. REED†

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INTRODUCTION

The present investigation was undertaken for the purpose of studying seasonal changes in the soil moisture and their relationship to certain phases of the activity of the trees. The work to be reported is a part of a major study on the 'Winter Injury' or 'Die-Back' of the Persian walnut (*Juglans regia*), certain phases of which were presented in 1919 (Batchelor and Reed<sup>1</sup>). The present publication will present data on the seasonal variations in soil moisture and on various factors which affect soil moisture.

We shall endeavor to show the extreme degree to which orchard soils in semi-arid regions may become desiccated at the end of the growing season, and to point out how this dry condition may persist during winters of light rainfall.

The M. Steinburg walnut grove lying one mile south of Hemet, Riverside County, California, was chosen for this study. At the beginning of the experiments, in the fall of 1918, this grove was typical of many which had suffered severely from winter injury. The

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grove is composed of seedling Santa Barbara softshell trees planted 44 feet apart each way. The trees were twelve years old in the spring of 1918. This grove received no irrigation until the fall of 1918. The development of a young walnut grove under dry-farm conditions is rather exceptional in the Hemet region, and the lack of irrigation may possibly be the reason why the grove under discussion exhibited an extreme case of winter injury.

The soil is an alluvial fine sandy loam with a low organic content. In general the subsoil is somewhat finer than the surface soil. This change in the fineness of the soil is gradual and perceptible to a depth of 12 feet. A well drilled in the grove soon after the experiment started showed the nearest ground water to be 84 feet from the surface.

CLIMATIC FACTORS AFFECTING SOIL MOISTURE

RAINFALL IN THE HEMET REGION, AND PENETRATION OF RAIN AND IRRIGATION WATER

The walnut grove in which the observations were made is located about four miles from a station of the U. S. Weather Bureau at San Jacinto. Records of rainfall kept since 1886 are available and may be used, although the precipitation at the walnut grove is, on account of neighboring topography, possibly less than at the official Weather Bureau Station. The records of rainfall referred to on the following pages were taken one mile from the grove by the Lake Hemet Water Company during the progress of the experiment. The mean seasonal precipitation for the twenty-seven years from 1892-93 to 1918-19, inclusive, is 13.2 inches, with a range of between 7.9 and 18.9 inches. The monthly distribution of rainfall is shown in table 1.

TABLE 1  
MEAN MONTHLY RAINFALL AT SAN JACINTO (MEAN OF 26 YEARS)

	Inches		Inches
July.....	.13	January.....	2.97
August.....	.21	February.....	2.28
September.....	.17	March.....	2.71
October.....	.66	April.....	1.12
November.....	1.07	May.....	.42
December.....	1.42	June.....	.03



The mean number of days on which there was precipitation of .01 inch or more at San Jacinto is 37. December had an average of four days on which rain fell, January and March had an average of seven, and February of six.

We may summarize the rainfall situation by saying that the grove under consideration is located in a semi-arid district which receives a mean seasonal rainfall of about 13 inches, most of which usually falls in the months of December, January, February, and March. Irrigation is practiced during the growing season, although this particular grove was not irrigated until the autumn of 1918, at which time the trees were twelve years old.

#### EVAPORATING POWER OF THE AIR

##### 1. Sunshine.

The duration of sunshine is related to loss of water in at least two important ways, the concomitant effect of heat and the use of water in photosynthesis. The Hemet region is characterized by almost complete freedom from clouds or fog during the growing season, and the ratio of sunshine received to the amount possible is high.

##### 2. Temperature.

This walnut grove is situated in a district characterized by high summer temperatures and a high evaporation rate. The summer temperatures are somewhat higher than those prevailing in the large walnut districts nearer the Pacific coast. Frequent comparisons of temperatures in the grove with those given by the official observer at San Jacinto showed little difference between the two. We may therefore refer to the official thermometric readings at San Jacinto, where observations have been taken for more than sixteen years. Some of the important temperature data are summarized in table 2, which gives monthly and annual averages together with the highest and lowest temperatures recorded in twenty-three years.

These figures show that the prevailing temperatures are in a way characteristic of conditions in southern California, although somewhat higher than those commonly found in other walnut-growing districts. The determination of the 'mean temperature,' either for the month or for the year, is of little importance in this locality because of the characteristically wide ranges of the daily temperatures. We may, therefore, consider the monthly maxima and minima.

The monthly maximum temperatures are rather high throughout the year, especially during the summer months. In June and the three following months the maximum temperatures are 15 to 20° higher than in the coastal regions of southern California. When the temperature rose to 115° in June, 1917 (the highest temperature ever recorded in this region), there was a small amount of burning on the leaves and young growth of walnut trees. Temperatures of 108° and 110° during the summer were frequently recorded by the instruments

TABLE 2  
TEMPERATURE DATA FROM UNITED STATES WEATHER BUREAU RECORDS AT  
SAN JACINTO (IN DEGREES FAHRENHEIT)

Month	Mean tempera- ture	Mean max. tempera- ture	Mean min. tempera- ture	Mean range	Highest tempera- ture	Lowest tempera- ture
January.....	48.8	65.2	35.8	29.4	90	7
February.....	52.2	68.0	37.2	30.8	93	19
March.....	54.9	71.0	40.4	31.6	102	23
April.....	59.5	77.3	44.7	32.6	101	27
May.....	63.8	80.0	48.3	31.7	109	32
June.....	71.4	90.9	53.8	37.1	115	37
July.....	76.8	96.8	59.2	37.6	111	44
August.....	76.4	96.6	58.4	38.2	109	43
September.....	71.6	91.8	53.6	38.2	110	38
October.....	64.2	82.3	47.2	35.1	103	30
November.....	56.8	74.4	39.8	34.6	99	21
December.....	50.3	66.2	34.4	31.8	89	20
Annual.....	62.2	80.0	46.0	34.0	.....	.....
No. of years observed.....	27	16	16	16	23	23

kept in this grove in standard U. S. Weather Bureau shelters. These individual high temperatures have little influence on the monthly averages, especially when several years are averaged together. In individual years the monthly averages may be somewhat higher than those given in table 2; for example, in July, 1920, the mean monthly maximum in this orchard was 103.5° F. There was no damage to the trees as a result of this high temperature. The mean minimum temperatures by months are from 30° to 40° below the maxima for the corresponding time. Indeed, the wide ranges in daily temperatures are very characteristic of these semiarid regions. On occasional days the temperature range may be as great as 50° F. This fall of temperature during the night is an important factor in the activity



of all vegetation, especially of arborescent plants, because it gives an opportunity for equilibrium to be reëstablished throughout the tree, after the depletion of water from the transpiring parts during the day. During the hottest part of the day the transpiration may be so rapid from the upper parts of the tree that the water-conducting system is unable to supply enough water to equalize the loss. The lower temperatures during the night, by diminishing the rate of transpiration, are favorable to a more uniform distribution of water throughout the tree.

The data in the last two columns of table 2 show the extremes of temperature which have been registered by the San Jacinto observer over a period of twenty-three years. It is evident that there are days even during the winter when the temperature is high enough to cause rapid evaporation during a few hours each day. Observations reported in another paper<sup>1</sup> showed that these trees do often suffer from desiccation during winters of scanty rainfall. The evaporation through the thin cortical layers of the young twigs is great enough under such conditions to reduce their water content to a serious degree and may result in their death. The injury from this cause is unduly severe if, as often happens, these high temperatures occur simultaneously with periods of low humidity and high wind velocity. The chart on page 12 of the publication cited<sup>1</sup> illustrates a condition which frequently occurs in walnut groves where the amount of water in the soil during the winter is dangerously near the critical point. The walnut trees near the flume which received more irrigation water during the summer suffered least from 'die-back.' The injury was progressively more severe as the distance from the flume increased, because the amount of soil water was progressively less.

### 3. Humidity.

The Hemet district is characterized by low atmospheric humidity during most of the growing season. Under these conditions there is a rapid loss of water from the soil and trees. While the high evaporating power of the air is conspicuous to the most casual observer, and its effects are quickly noticed, it is difficult to make quantitative measurements of the condition which is commonly expressed by the term 'aridity.'

We know of no determination of the evaporation of water from a free surface in the Hemet district, but we may gain a fairly trust-

worthy idea of its rate from the data published by Russell.<sup>5</sup> His tables show evaporation of 43.3 inches at Sacramento and 56 inches at Fresno during the eight months from April 1 to November 30. If we assume that the evaporation at Hemet is 48 inches for this period, we shall probably not be far wrong. During this period the average rainfall for the Hemet-San Jacinto region is 3.81 inches. Using these

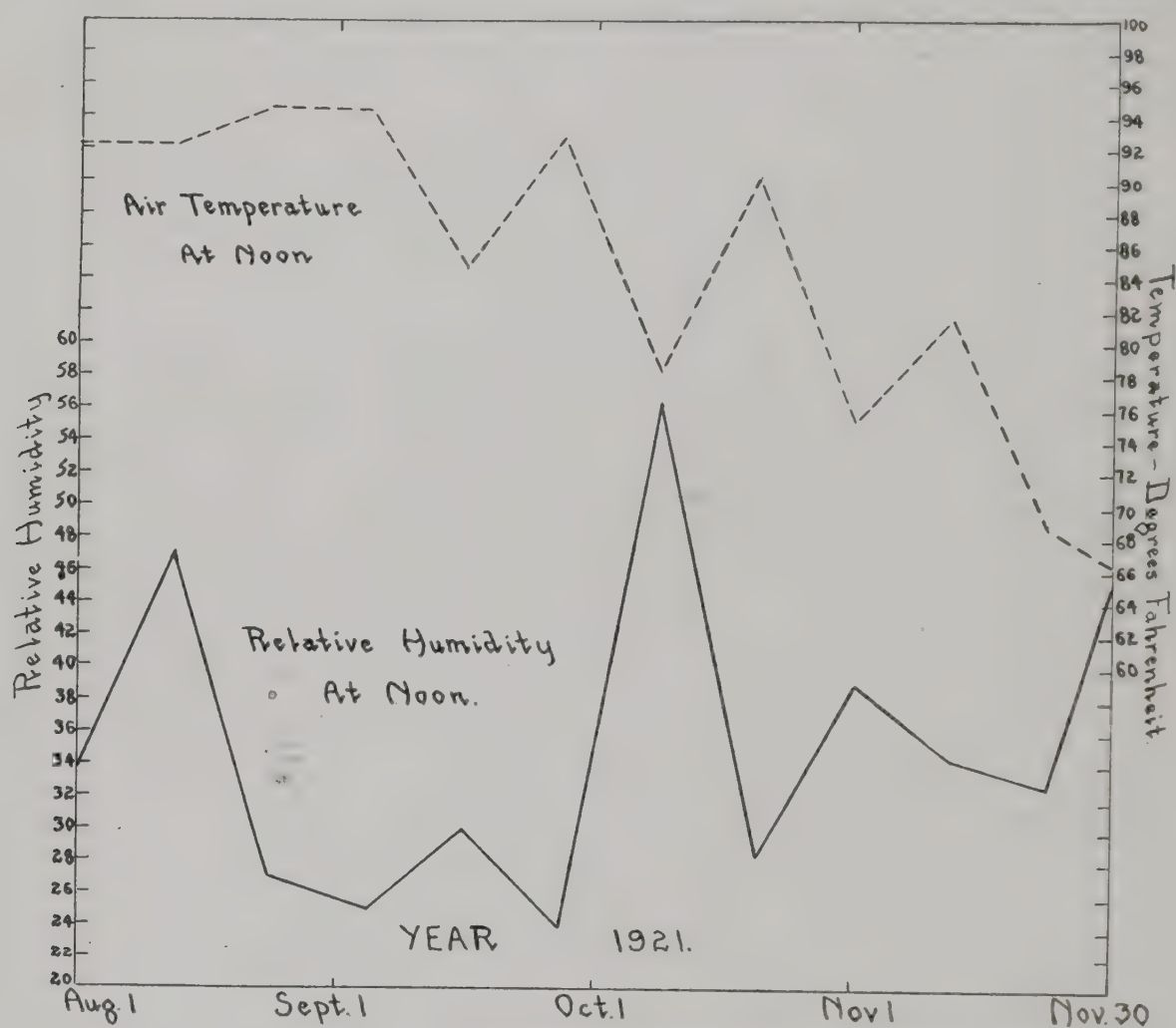


Diagram 1. Relative humidity and temperature at Hemet, August 1 to December 1, 1921.

figures, we see that the ratio of rainfall to evaporation is .08. This period, including two-thirds of the calendar year, is certainly marked by high evaporation and shows one aspect of the climatic conditions which govern plant life in this region.

Determinations of the vapor pressure of the air at Hemet were made during the latter part of the season of 1921 by means of wet and dry bulb thermometers. The determinations are expressed as mean relative humidity for ten-day intervals and are shown in diagram 1. They show that, for most of the season subsequent to



July, the moisture content of the air was very low. The one outstanding exception occurred early in October. It followed a precipitation of 2.95 inches of rain from September 30 to October 2, and was simultaneous with a depression of the mean midday temperature. Another rain on October 23 and 24, which gave a precipitation of .43 inch, also caused an increase in the relative humidity and a fall in mean midday temperature.

These figures give a picture of the evaporating power of the air at a time of the day when evaporation is probably close to the maximum. It is certain that the rate of evaporation during most of the day is much below this figure. During the night the trees have an opportunity to recover from water deficit incurred during the middle of the day.

#### 4. Winds.

Winds in the Hemet region have a well-marked influence upon evaporation. During the summer and fall months there is usually a moderate westerly wind every day. This is the 'coast breeze' which blows from the Pacific Ocean to the hot interior deserts. Although the wind comes from the ocean, its relative humidity is not high, at least by the time it reaches the Hemet district, which is 45 miles from the coast. This daily coastal wind undoubtedly increases the evaporation from both soil and trees.

Evaporation is also increased by irregular east or north winds which come for periods of several days during the fall or early winter months. These winds, coming from the inland deserts, are powerful desiccating agencies. The relative humidity often ranges from 13 to 20 per cent for twenty-four to forty-eight hours at a time, during the prevalence of these northerly winds. Although the trees are partially or wholly bare of leaves at this season, there is nevertheless an appreciable loss of water through the thin bark of the young growth.

#### LENGTH OF GROWING SEASON

The time between the last killing frost in the spring and the first killing frost in the fall, generally designated as the 'frostless season,' corresponds fairly closely with the growing season for walnuts.

The Weather Bureau records show that the average length of the frostless season at San Jacinto for eighteen years is 254 days, with a

minimum of 199 and a maximum of 301 days. For the three years during which our observations were being made in this orchard at Hemet the dates between killing frosts were:

1919, March 21 to November 28 (252 days)

1920, March 27 to December 4 (252 days)

1921, April 6 to November 18 (227 days)

The length of the first two seasons was very nearly the mean, but the last season was considerably shorter.

### CONDITION OF TREES AND SOIL AT THE BEGINNING OF THE EXPERIMENTS

The majority of the trees in the several plots (diagram 2) were severely killed back by winter injury during the winter of 1917-18. That the cause of the winter injury in this grove during this season was due to winter drought seems reasonably certain. The first winter rain occurred January 13, 1918, when .43 of an inch fell. The second rain of .25 of an inch occurred on January 25, 1918.

The last rain of 0.3 of an inch or more preceding that of January 13, 1918, fell on April 17, 1917 (with the exception of a thunderstorm on July 27 which gave 1.87 inches). Thus 270 days had elapsed between rains that were of sufficient amount to affect the moisture content of the soil in any great portion of the root zone. The shower in July probably reached a few of the surface roots in the first foot of soil, which was in an air-dried condition previous to the rain. Not until January 26, 1918, did enough rain fall to moisten the surface foot of soil. On this date 1.14 inches of rain fell.

The extreme dryness of the soil in this grove before the winter rains began can be realized when it is recalled that there were twenty-two walnut trees per acre, twelve years old, growing on the land under dry-farm conditions where the normal rainfall of 13 inches comes almost exclusively during the dormant period of the walnut tree. Thus in the absence of rain from July to January 13, the soil-moisture content of the root zone was reduced to a point seldom reached under cultural conditions.

Soil samples were taken October 30, 1917, when the leaves on the trees were turning yellow and a portion of them had fallen. At this



time the surface foot of soil was dust-dry and showed a moisture content of only 0.7 per cent. The condition of the subsoil is shown by the ratio given in table 3.

As mentioned above, such dryness of the subsoil as herein reported is seldom observed under cultural conditions. In fact the results of the preliminary observations were so extreme that it seemed as though an unnoticed error must have occurred in weighing or calculating. Repetitions, however, confirmed these findings.

TABLE 3

THE SOIL MOISTURE AND ITS RELATION TO THE HYGROSCOPIC COEFFICIENT AT THE  
END OF THE 1917 GROWING SEASON

Depth	Hygroscopic point	Moisture observed	Ratio
	Per cent	Per cent	
2nd foot.....	2.44	1.31	0.54
3rd foot.....	2.26	1.74	0.77
4th foot.....	2.74	2.34	0.85
5th foot.....	4.30	2.97	0.69

Most of the soil-moisture studies made by previous workers under field conditions have dealt with that portion of the soil moisture which is above the wilting point, because they have not been made under the extremely arid conditions which may be observed in regions like that here described. Alway,<sup>2</sup> however, has reported studies in which the soil moisture was below the hygroscopic point. He gives data upon the soil of an abandoned olive orchard in Arizona, of the prairies of southwestern Nebraska,<sup>3</sup> and of cylinders in which desert legumes had been grown under controlled conditions. Regarding the condition of these perennial desert legumes when the observations were made the author reports, "In none were all the plants dead when the cylinders were opened but in the case of each all the tips had died and nearly all the leaves had fallen, the most vigorous plant in each retaining only from five to seven compound leaves." "In experiments with perennial desert legumes the plants remained alive after the water content had fallen slightly, but distinctly, below the hygroscopic coefficient . . . ." Apparently the moisture conditions in the dry-farm walnut grove are similar to those in Alway's cylinders in which desert legumes were grown. As that author has suggested in the case of the desert plants, although there is no evidence of any ability on the

part of the walnut to use the last portion of free water *for growth*, there is an indication that the moisture between the wilting point and the hygroscopic point, and even some of the water below the



Fig. 1. A general view in the Steinburg walnut grove in May, 1918, showing the injury which resulted from a lack of soil moisture during the preceding winter.

hygroscopic point, may have a very high value for the maintenance of the life of the perennial and tree crops.

As heretofore noted, in the spring following the dry winter of 1917 many of the trees were dead in the uppermost branches and some of them killed back nearly to the main scaffold limbs. The distribution of the injured trees in the grove is given in diagram 2, while the nature of the injury on some of the individual trees is shown in figures 1 and 2.



Fig. 2. A badly injured tree in the Steinburg grove, showing how the young branches tend to die back as a result of winter drought.



The grove was dry-farmed during the summer of 1918 which followed a total rainfall of 13.46 inches for the entire rainy season preceding. In the following November the owner installed an irrigation pipe line and the grove was given a light irrigation of 3.6 acre

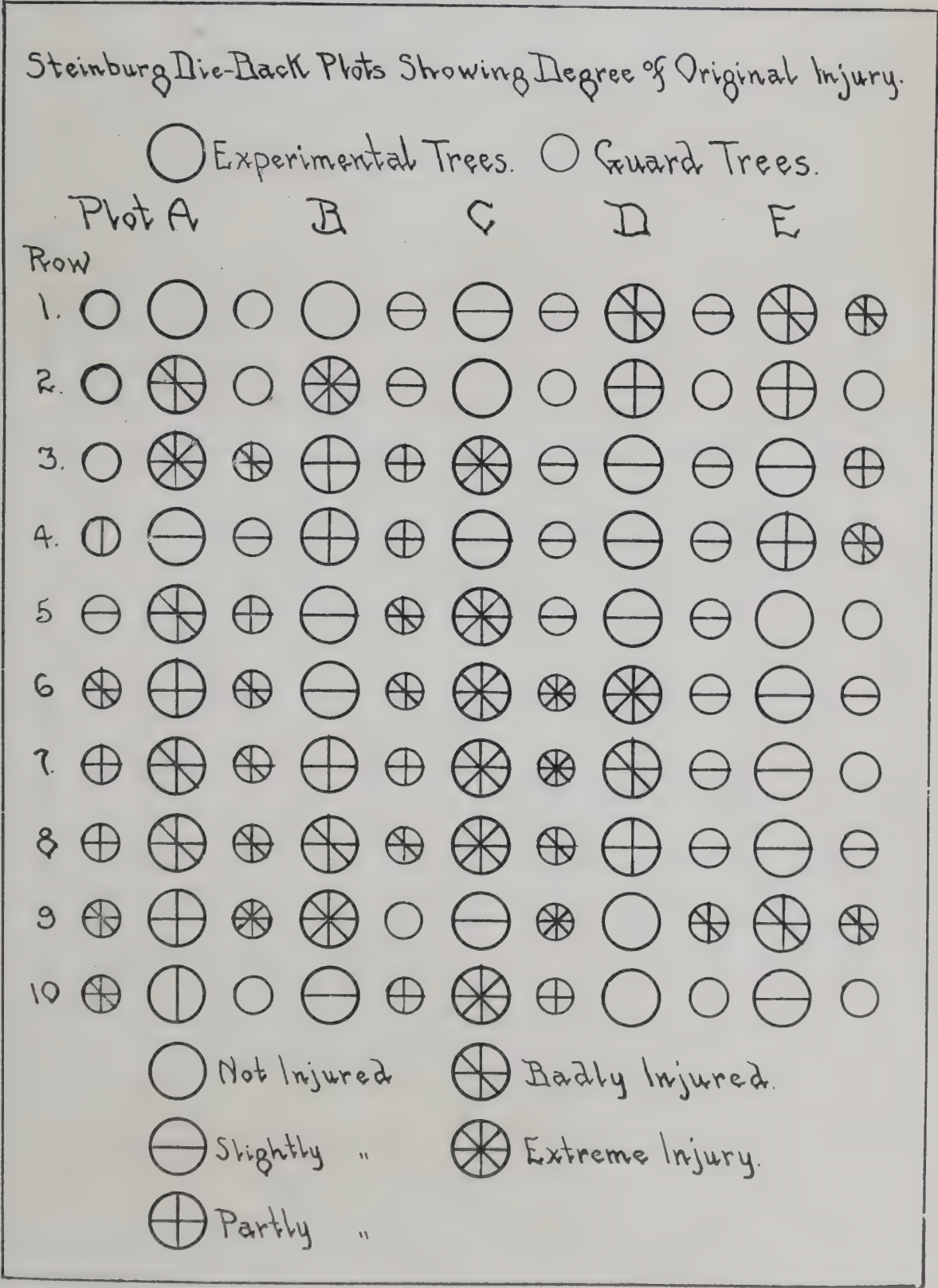


Diagram 2.    Distribution of injured trees in Steinburg grove, 1918.

inches per acre, after which it passed under the control of the Citrus Experiment Station and was irrigated as will be described later.

Since the winter of 1917-18 the rainfall has commenced early and has been sufficient in amount to prevent drought injury; thus the seasons have not been favorable to a study of extreme winter drought conditions as related to the growth of winter-irrigated walnuts. This may therefore be a somewhat incomplete report of progress. Inasmuch, however, as the work is to be discontinued on the Steinburg grove and taken up on the Citrus Experiment Station grounds, it seems best to report progress to date.

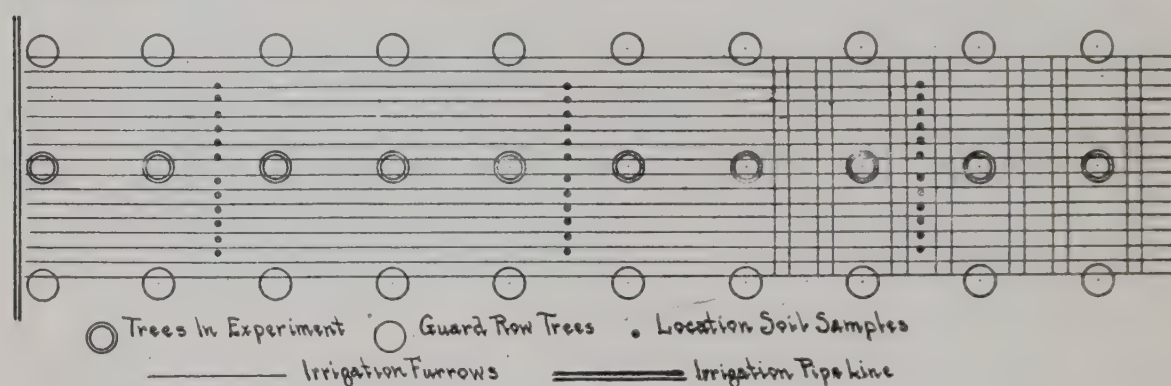


Diagram 3. Map of a typical plot showing location of irrigation furrows, locations from which samples were taken, and relative positions of experimental and guard trees. .

## METHODS OF PROCEDURE

### PLAN OF PLOTS

Each trial plot was laid out to include ten trees in a single row with guard rows between plots. The entire area from guard row to guard row was irrigated, sampled, and in every way regarded as belonging to the adjacent experiment row. See diagram 3.

### IRRIGATION

The irrigation water was measured over a rectangular weir and applied in deep furrows 440 feet long; 8 furrows were used in each interspace of 44 feet, with the lower portion of the plot cross-furrowed. The method of cross-furrowing retarded the velocity of the water and increased its depth in the furrows, thus largely equalizing the amounts of water received by the upper and lower ends of the plots.



Water was applied at the rate of 15 southern California miner's inches (0.3 of a second foot) per acre. This is a larger stream of water per acre than is commonly used in the Hemet-San Jacinto section. Because of the sandy nature of the experimental plots, however, the use of the stream mentioned was in harmony with good irrigation practice.

TABLE 4

IRRIGATION WATER APPLIED TO THE PLOTS (IN ACRE INCHES PER ACRE)

Dates	Plots				
	A	B	C	D	E
<i>1918</i>					
November 8-9.....	3.6	3.6	3.6	3.6	3.6
December 18.....	5.7		4.0		9.7
<i>1919</i>					
April 16.....		1.8			
May 14.....		1.8			
June 19.....	4.2	1.8	4.2	4.2	4.2
July 16.....	4.2	1.8		4.2	4.2
August 13.....	4.2	1.8	4.2	4.2	4.2
September 17.....		1.8			
October 15.....		1.8			
December 11-13.....	6.6		4.2		9.7
	28.5	16.2	20.2	16.2	35.6
<i>1920</i>					
March 16-17.....	7.2				
April 20.....		1.8			
May 18.....		1.8			
June 15.....	4.2	1.8	4.2	4.2	4.2
July 20.....	4.2	1.8		4.2	4.2
August 10.....	3.2	1.8	4.2	4.2	4.2
September 15.....		1.8			
October 13.....		1.8			
November 30.....	13.1		4.2		9.7
	31.9	12.6	12.6	12.6	22.3
<i>1921</i>					
April 18.....		1.8			
May 16.....		1.8			
June 14.....	4.2	1.8	4.2	4.2	4.2
July 19.....	4.2	1.8	4.2	4.2	4.2
August 9.....	4.2	1.8		4.2	4.2
September 13.....		1.8			
October 11.....		1.8			
December 13-15.....	6.3		4.2		6.5
	18.9	12.6	12.6	12.6	19.1

In general the water-holding capacity of this soil gradually increases with the depth. The hygroscopic point of the surface soil varies from 2 to 3 per cent, whereas that of the sixth and seventh foot varies from 3 to 6 per cent. There is one abrupt change in soil type in plot D. The fourth foot in one of the transverse sampling areas consists largely of coarse sand the hygroscopic point of which is only 1.98 per cent.

Since the experiment was installed for the purpose of acquiring information on the relation between soil water and die-back, the amounts of water applied to the several plots and the time of their application were varied. The schedule of applications is given in table 4. It will be seen that this plan of irrigation afforded an admirable opportunity to observe the water content of the soil as affected by the growth of the trees and by the application of different quantities of water. Plots B, C, and D in the last two years received the same total quantity of irrigation water (12.6 inches), the customary amount in the Hemet district, but the time of application was varied in the different cases. Plots A and E were given considerably more water in the winter than the other plots.

So far as the trees are concerned, however, the amount of water in the soil is of more importance than a record of the amount of water applied. Determinations of the amount of water in the soils of the various plots were made before and after each irrigation and at such other times as were judged necessary.

#### SOIL SAMPLING

As noted in diagram 3 the samples were taken across the upper, middle, and lower end of each plot. At each transverse area samples were taken for each foot separately, down to and including the seventh foot, and each sample consisted of a composite made up of six cores from the soil tube.

A moisture determination was made on each of the composite samples and the three results were averaged to obtain the mean moisture content for each footlevel of the plot.



## METHOD OF EXPRESSING RESULTS

## 1. Soil Moisture Data.

In the following discussion the soil moisture content is stated in the form of a ratio to the hygroscopic coefficient, following the usage of Alway and co-workers,<sup>3</sup> whose comprehensive tables and discussions might well serve as an example to other workers. With a tabulation of the hygroscopic coefficients in tables 7 and 8 preceding the respective ratios, the reader may visualize the type of soil worked with as well as its relative moistness.

We have usually compared our data on the moisture content of the soils with the hygroscopic coefficient rather than the wilting-point coefficient. There seemed to be two good reasons for such a procedure: first, the trees often did not wilt when the soil moisture in the root zone was below the 'wilting point,' and second, the comparisons of moisture content with the wilting point would frequently involve the use of minus quantities.

## 2. Reliability of the Hygroscopic Coefficient.

The labor involved in sampling the several plots twice per month throughout most of the year made the determination of the moisture equivalent and hence of the hygroscopic coefficient for each respective composite of six cores a practical impossibility. The hygroscopic coefficients here presented are the mean values for a series of six determinations made in the respective areas, each determination representing a composite sample taken entirely independently of every other at an interval of at least thirty days. The determinations for the six respective samples were made by four different people. The personal and time elements, as well as the error in sampling, were therefore somewhat reduced in obtaining this mean as compared with the determination of six samples from the same composite, or of samples from different composites taken by the same person simultaneously. The six determinations may thus be considered as six attempts to measure the same thing, viz., the hygroscopic point of a certain foot section of a given plot.

A statistical inquiry may be profitably applied to these data to test their reliability as compared with the mean of an infinite number of determinations. The tables of 'Student'<sup>6</sup> serve as a ready means of applying statistical methods to this inquiry. As this author so aptly states: "Any experiment may be regarded as forming an indi-

vidual of a population of experiments which might be performed under the same conditions. A series of experiments is a sample drawn from this population." With this viewpoint in mind it becomes of interest to determine the reliability of a mean of a series of six determinations of the hygroscopic coefficient. The third foot in plot A in the sampling area nearest the pipe line will serve as a fair example. Following 'Student's' procedure the example works out as follows:

RELIABILITY OF THE MEAN OF THE HYGROSCOPIC COEFFICIENT

V	D	D <sup>2</sup>
3.91	.33	.1089
3.80	.22	.0484
3.06	— .52	.2704
3.24	— .34	.1156
3.89	.31	.0961
3.57	— .01	.0001
M = 3.58		6) .6395
		.1066

$$S. D. = \sqrt{.1066} = .3265$$

The probability that the true mean does not differ from the mean of the sample by more than  $\pm .3$  per cent moisture may be found as follows:

$$Z = \frac{.3}{.3265} = .9$$

In Student's tables when  $Z = .9$  and  $N = 6$ ,  $P$  is .9498. Subtracting,  $1.0000 - .9498 = .0502$ , which may be taken to mean that the chances are .9498 : .0502 that the true mean does not exceed the mean of the sample by more than .3 per cent moisture.

In our problem, however, we are concerned with a probability that the true mean is just as liable to lie below as above the mean of the sample. Thus we proceed farther to obtain these odds as follows:  $.0502 \times 2 = .1004$ . Subtracting as before,  $1.0000 - .1004 = .8996$ . The odds that the true mean does not vary from the mean of the sample by more than  $\pm .3$  per cent are .8996 : .1004, or practically 9 to 1.

Although odds of 9 to 1 are not considered a 'reasonable certainty' in statistical studies, such odds do indicate that the use of the mean of the hygroscopic coefficients is probably as reliable within the limits of  $\pm .3$  per cent moisture as most comparisons, subject to the error of soil sampling.



RESULTS AND DISCUSSIONS

PENETRATION OF RAIN AND IRRIGATION WATER

The soil on which this grove is located absorbs water readily, and there is practically no run-off, even during seasons of heavy precipitation. The rate of water penetration is roughly indicated by table 5,

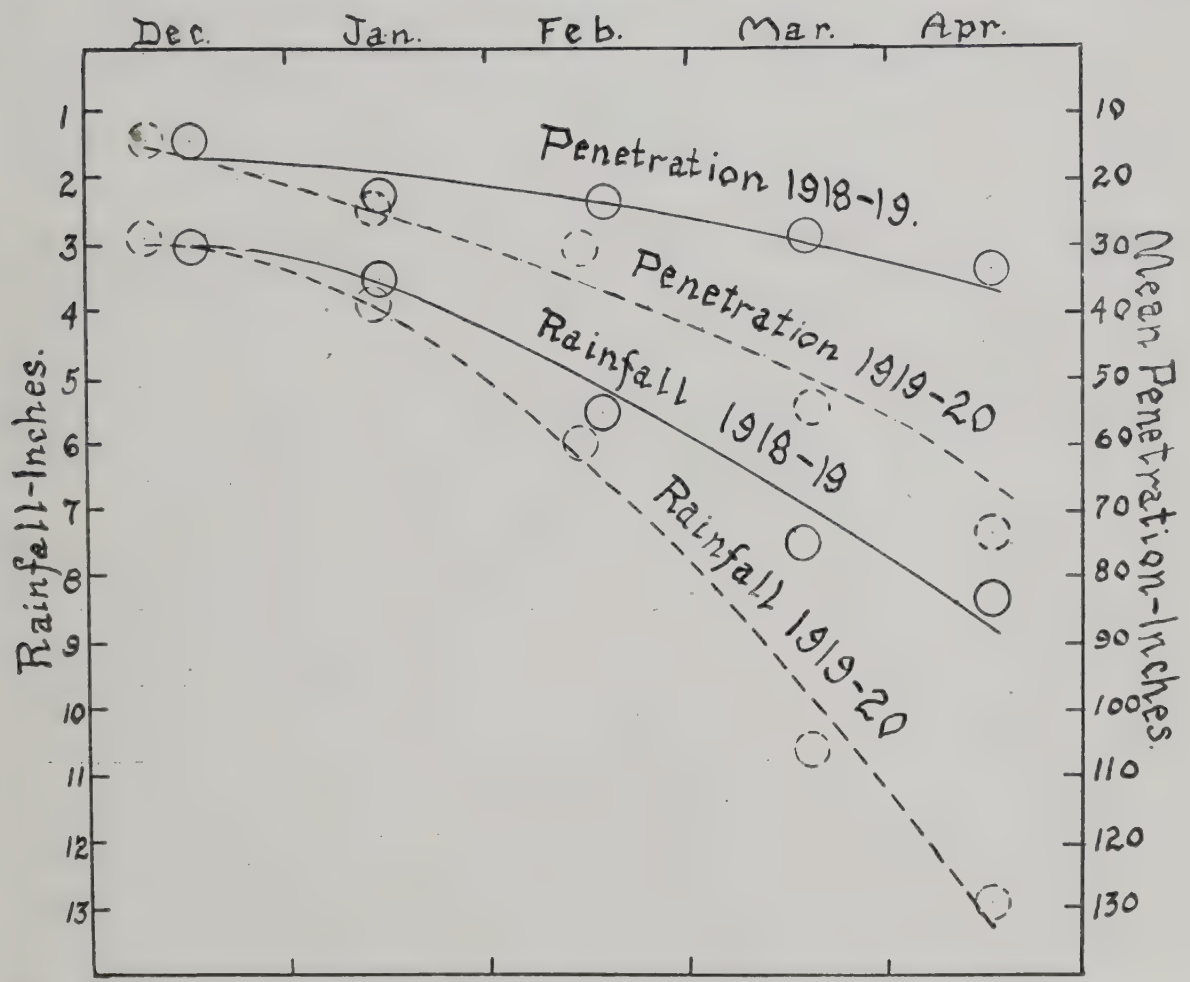


Diagram 4. Relation of rainfall to its penetration in the grove in two seasons.

showing the observations made during the winters of 1918-19 to 1920-21, inclusive. Diagram 4 represents the relation of rainfall to penetration as based on two-years' observation.

The moisture penetrates this soil from 4 to 6 inches for each inch of rain. The factors responsible for this relatively small penetration seem to be: first, the extreme initial dryness of the soil; second, the small amount of rain falling in many individual storms; third, a rate of evaporation between storms so rapid that much of the water passes into the air before another storm occurs.

The penetration of irrigation water may be illustrated by plot A as observed during the winter of 1919-20. The rain to December 9 was 2.87 inches and it had penetrated 10 inches on plot A. The plot was then irrigated with 6.56 acre inches per acre, and 8 days later the moisture penetration in 18 holes averaged 54 inches. The soil moisture had been lowered, therefore, 6.7 inches per acre inch of water applied. This is somewhat less penetration than Harding'

TABLE 5  
RAINFALL AND DEPTH OF PENETRATION IN PLOTS B AND D (IN INCHES)  
1918-1919

Date	Dec. 16	Jan. 15	Feb. 17	Mar. 18	April 16	Average penetra- tion per inch of rainfall
Rainfall to date.....	2.99	3.45	5.46	7.47	8.25	
Penetration in dry plots..	14	22	23	28	33	4.0
		1919-1920				
Date	Dec. 9	Jan. 13	Feb. 18	Mar. 18	April 19	
Rainfall to date.....	2.87	3.91	5.96	10.58	12.88	
Penetration in dry plots ..	10	24	30	58	73	5.7
		1920-1921				
Date	Dec. 15		Feb. 2	Mar. 8	April 18	
Rainfall to date.....	1.63	.....	4.60	5.12	6.20	
Penetration in dry plots ..	18	.....	24	25	37	5.9

Mean 5.2

reports for a similar amount of water. Harding, however, was dealing with movement of soil moisture under ordinary irrigation practice where the content seldom drops below the wilting point, whereas we are considering the winter irrigation of a soil in which the initial soil moisture was close to the hygroscopic point 120 days after the last irrigation.

The summer irrigation which was given at a time when the moisture was close to the wilting point penetrated deeper and harmonizes



more closely with Harding's observations. This is best illustrated by plots A and C following August irrigation of 1920. Table 6 shows the amount of water applied, the relative dryness of the soil before irrigation and the average penetration per acre inch of water. In plot A, with the moisture of the first 60 inches of soil considerably above the wilting point, the average penetration of 3.18 acre inches of water was 55.9 inches or 17.6 inches per acre inch of irrigation water. Pot C, which was much dryer and in fact had a moisture content nearer the hygroscopic point than the wilting point, after an irrigation of 4.2 acre inches, showed an average penetration of 43.3 inches or 10.3 inches per acre inch of irrigation water.

TABLE 6

DEPTH OF PENETRATION OF IRRIGATION WATER ON PLOTS A AND C, AUGUST, 1920

Plot	Water applied	Soil moisture before irrigation (upper 60 inches)	Average penetration per acre inch
	Acre inches* per acre	Ratio to hygroscopic point	Inches
A.....	3.18	1.66	17.6
C.....	4.20	1.17	10.3

\* Amount of water accounted for by rise in moisture content of soil 10 days after irrigation.  
A 2.128 acre inches per acre.  
C 2.641 acre inches per acre.

It may be instructive to examine the records of plot A and to note how the applications of water affected the moisture content of the soil to the depth of 7 feet. Diagram 5 represents graphically the per cent of water in the soil in one-foot sections from December, 1919, to the end of October, 1920, expressing the water as a per cent of the weight of dry soil. Between August 1 and December 1, the precipitation amounted to 1.84 inches at Hemet, but it had fallen in scattering showers and had not appreciably affected the water content of the soil below the first foot. At the beginning of the observations the soil moisture below the first foot was very close to the hygroscopic coefficient. After the irrigation of 6.6 inches on December 11-13 there was a perceptible increase in the water content of the second, third, fourth, and fifth foot layers of soil, as determined from samples taken six days after irrigation. At the time the soil was next sampled, in March, the water content of the deeper layers had

increased to about the same per cent as that of the upper layers. This was due both to the steady downward movement of water and to the addition of 6.56 inches of rain after January 1. The heavy irrigation (7.2 inches) given on March 16 and 17 appreciably raised the water content of the deeper layers of the soil and to a less extent of the

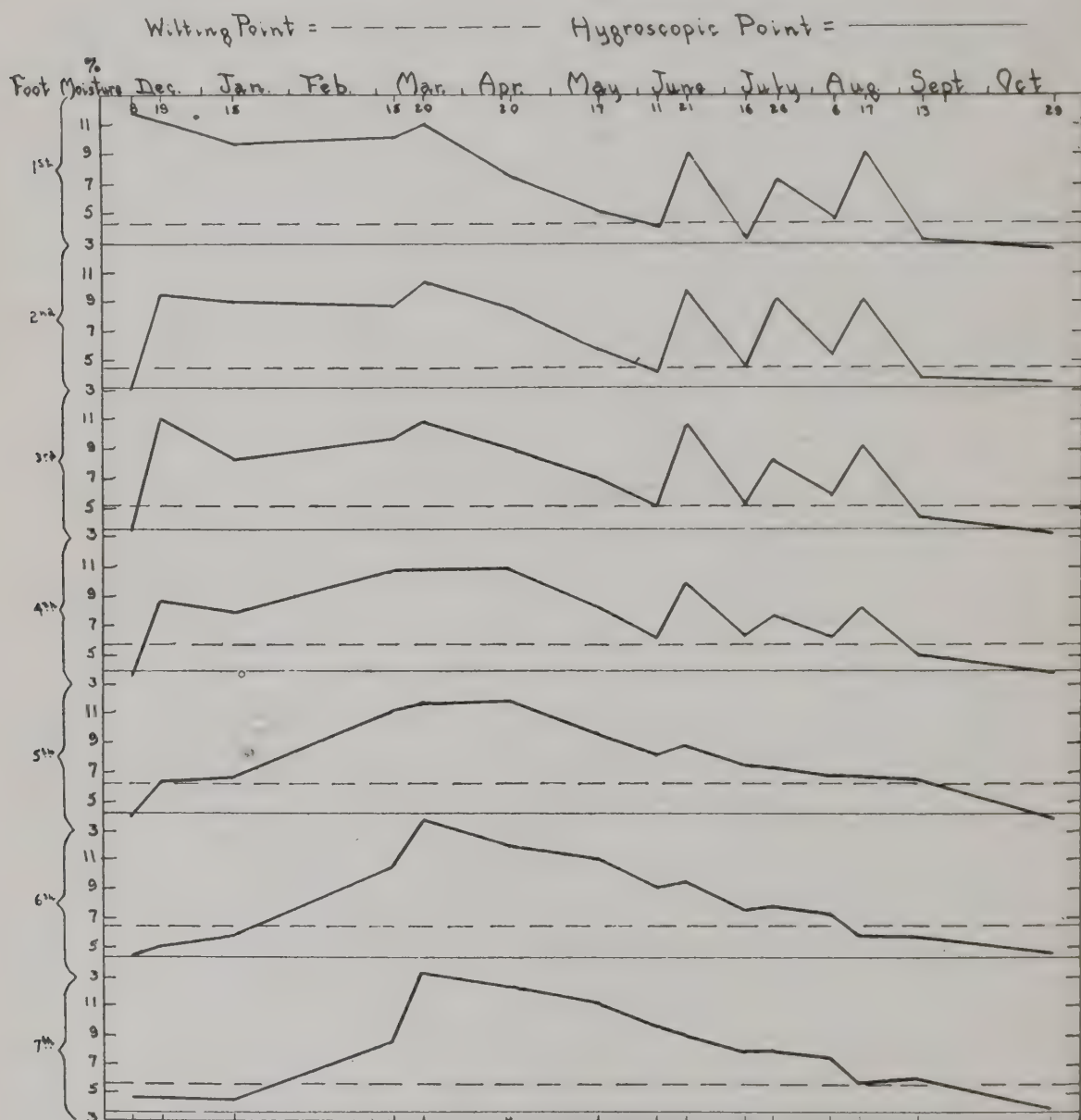


Diagram 5. Seasonal variations in moisture content of the upper seven feet of soil in plot A from December, 1919, to October, 1920. The horizontal line represents the mean hygroscopic coefficient of the samples at each level, and the broken line represents their wilting point coefficient.

surface layers. After a precipitation of .93 inches on March 23 there were no more rains of consequence and the soil water content steadily diminished until the next irrigation, owing to the removal of water by the trees during the period of rapid growth characteristic of spring and early summer. When sampled on June 10, the soil water of the



first four foot-layers was practically at the wilting point. In other words, the trees had removed practically all of the water 'for growth' from the zones in which most of the active roots are located. The application of 4.2 inches of irrigation water in June and July, and of 3.2 inches in August temporarily raised the water content of the first four foot-layers of soil, but made little difference in the water content of the lower layers.

The depth of penetration of like amounts of irrigation water varied from year to year according to the rainfall. This can be readily understood by noting the rainfall penetration shown in diagram 4.

#### EFFECT OF IRRIGATION ON SOIL MOISTURE

The relation of soil moisture during the winter months to the need of irrigation the following summer or the following growing season is of both practical and scientific interest. Table 7 gives the moisture present in plots A and D from March, 1920, at monthly intervals until October. Both plots received practically the same irrigation during June, July, and August. Plot A received winter irrigation of 13.8 acre inches per acre, while plot D was not winter-irrigated. The winter rains amounted to 13.74 inches and penetrated 73 inches on plot D. Although plot A showed a considerably greater amount of soil moisture at the beginning of the growing season, there was no significant difference in the two plots by the middle of July. This table and table 8 show that there is no 'hold-over' of soil moisture from one season to another in the zone of greatest root development. It may also be concluded from this table that the effect of heavy winter irrigation will not persist beyond the middle of the growing season under the condition in this orchard. From this time until the beginning of the dormant period, the irrigation needs are the same as though no winter water had been applied. This may not apply to the subsoil where relatively few roots are developed, a depth from 8 to 20 feet from the surface, provided any of the irrigation water reaches such depths; the rainfall seldom reaches a depth of more than 4 or 5 feet in the district in question. Slight irregularities had gradually developed in plots A and D when the data in table 7 were taken. Since the former plot had been heavily winter-irrigated for the two years previous, the trees had grown somewhat larger and no doubt made somewhat greater demands on the soil moisture than those in plot D.

TABLE 7

COMPARISON OF SOIL MOISTURE IN PLOT A RECEIVING HEAVY WINTER IRRIGATION OF 13.8 ACRE INCHES PER ACRE  
AND PLOT D WHICH WAS NOT WINTER-IRRIGATED IN 1919-1920

Soil moisture expressed as a ratio of the hygroscopic point

Ft.	Hygro. point		March			June			July			August			September			October		
	A	D	A	D	Dif.	A	D	Dif.	A	D	Dif.	A	D	Dif.	A	D	Dif.	A	D	Dif.
1.....	3.05	2.45	3.67	3.30	0.37	1.39	1.08	0.31	1.11	0.94	0.17	1.61	1.45	0.16	1.12	0.96	0.16	0.92	1.11	-0.19
2.....	3.17	2.43	3.41	3.08	0.33	1.40	1.34	0.06	1.47	1.66	-0.19	1.74	1.87	-0.13	1.24	1.37	-0.13	1.12	0.95	0.17
3.....	3.61	2.83	3.05	3.17	-0.12	1.43	1.71	-0.28	1.50	1.72	-0.22	1.67	1.91	-0.24	1.26	1.51	-0.25	0.94	1.08	-0.14
4.....	3.97	2.77	2.78	3.17	-0.39	1.57	1.55	0.02	1.61	1.88	-0.27	1.64	2.08	-0.44	1.28	1.52	-0.24	0.95	1.09	-0.14
5.....	4.21	4.56	2.80	2.40	0.40	1.95	1.63	0.32	1.78	1.63	0.15	1.64	1.78	-0.14	1.34	1.46	-0.12	0.92	1.02	-0.10
6.....	4.42	5.71	3.12	1.80	1.32	2.08	1.69	0.39	1.76	1.88	-0.12	1.69	1.83	-0.14	1.30	1.58	-0.28	1.06	1.07	-0.01
7.....	3.79	5.32	3.53	1.61	1.92	2.56	1.78	0.78	2.13	2.13	0.00	2.02	1.93	0.09	1.64	1.70	-0.06	1.03	1.33	-0.30
Mean	3.75	3.72	3.19	2.62	0.55	1.77	1.54	0.23	1.62	1.69	-0.07	1.72	1.84	-0.12	1.31	1.44	-0.13	0.99	1.09	-0.10



TABLE 8

SOIL MOISTURE IN THREE PLOTS EXPRESSED AS A RATIO OF THE HYGROSCOPIC POINT

(The hygroscopic points stated below are the means of six different sets of samples and determinations for the respective foot-samples.)

Depth of foot-section	Plot A				Plot B				Plot C			
	Soil moisture				Soil moisture				Soil moisture			
	Hygro. Point	Oct. 1919	Oct. 1920	Sept. 1921	Hygro. Point	Oct. 1919	Oct. 1920	Sept. 1921	Hygro. Point	Oct. 1919	Oct. 1920	Sept. 1921
Upper portion of plot	1	2.79	0.76	0.83	1.18	2.43	1.20	2.00	1.50	2.11	1.42	0.99
	2	2.98	1.03	1.15	0.92	2.77	0.88	1.78	1.09	2.39	1.04	0.94
	3	3.58	0.99	0.99	0.92	2.71	1.04	1.59	1.62	2.33	1.14	1.11
	4	4.09	1.06	1.01	0.90	3.39		1.33	1.23	2.69	0.90	1.36
	5	4.08	1.08	0.81	0.97	3.67	0.95	1.05	1.00	4.88	0.88	0.95
	6	3.85	1.02	1.05	0.98	3.38	1.15	1.32	1.17	6.42	0.98	1.06
	7	3.69	1.08	1.02	1.00	3.09	1.09	1.05	1.32	4.87	0.93	1.19
Middle portion of plot	1	2.85	0.46	1.15	0.95	2.52	0.61	1.50	1.11	2.52	1.23	0.46
	2	2.86	0.88	1.24	1.13	2.56	1.09	1.27	1.38	2.44	0.94	0.96
	3	3.25	1.06	1.17	1.12	3.05	1.06	1.10	1.22	2.26	0.99	0.91
	4	3.50	1.06	1.15	0.99	4.08	0.98	1.07	1.22	2.74	0.85	1.22
	5	3.97	1.06	1.05	1.08	4.24	0.92	1.00	1.07	4.30	0.92	0.97
	6	4.30	0.95	1.13	1.08	3.72	1.01	1.04	1.15	4.41	0.92	1.11
	7	3.46	1.10	1.21	1.25	3.88	0.80	1.03	1.13	3.35	1.12	1.11
Lower portion of plot	1	3.52	0.81	0.96	0.61	2.92	1.22	1.23	0.70	2.51	1.19	0.72
	2	3.67	0.88	1.45	0.88	3.04	0.89	1.03	1.26	2.60	0.94	0.83
	3	3.99	0.98	0.94	0.89	3.39	1.23	1.06	1.14	3.01	0.91	0.99
	4	4.31	0.97	1.01	0.91	4.28	1.14	1.09	0.81	4.48	0.95	0.85
	5	4.58	1.02	1.00	1.02	4.59	1.10	1.03	0.93	5.52	0.89	0.82
	6	5.10	1.12	1.06	0.98	4.88	1.07	0.96	0.99	3.68	1.03	0.98
	7	4.23	1.25	1.12	0.94	4.48	0.86	0.91	0.83	2.95	1.05	1.03
Mean		0.98	1.07	0.99			1.01	1.21	1.14		1.01	0.98

The percentage of water in the soil of plot A and its variations during the growing season are given in diagram 5. The water content of each of the upper seven feet of soil is stated in relation to the wilting and the hygroscopic coefficients. The diagrams show how the water content of soil lying at various depths is influenced by additions of water. The upper layers of soil respond more quickly to a fall of rain or to an application of irrigation water, but also lose their water more quickly. The loss of water during the summer months is principally due to the absorbing action of the tree roots, which are more densely distributed in the upper four feet of soil in this grove. It will be noted that the water content of each foot-layer except the first was below the wilting point on December 5, 1919; indeed, with the exception of the first and seventh, all were below the hygroscopic point. As the result of an irrigation of 6.6 acre inches per acre, the moisture content of the second, third, and fourth foot-layers rose sharply, but the fifth, sixth, and seventh foot-layers were not raised above the wilting point.

As a result of the winter rains and of another irrigation of 7.2 acre inches the water content of the various layers was much higher when sampled March 31, 1920. The increase was especially marked in the case of the first and the seventh foot-layers. Subsequent samples taken April 19, May 17, and June 11 showed a steady decline in water content. The trees put out new foliage and began active growth the last week in March.

The samples taken on June 11 showed that the water of the layers of soil occupied by most of the tree roots had fallen approximately to the wilting point. Irrigations of 4.2 acre inches per acre in June, July, and of 3.2 in August, materially raised the water content for a brief time, but within thirty days it dropped back to the approximate value of the wilting point. During the entire summer the water content of the fifth, sixth, and seventh foot-layers declined almost continuously, showing but little response to the irrigation water applied. No water was applied to this plot in September or October. When the plot was sampled on October 29, the soil at all levels in the upper seven feet was approximately at the hygroscopic point.

These observations show that in this grove the stored soil moisture from heavy winter rains or irrigation is of importance in the upper root zone only up to the middle of the growing season. The effect of



such moisture may persist in the subsoil where fewer roots are developed, until well past the middle of the growing season. They also show the failure of ordinary medium applications of irrigation water (4.6 acre inches per acre) to maintain the soil moisture during the late summer months.

#### DESICCATION OF THE SOIL BY TREE ROOTS

The dryness to which the soil is reduced\* at the end of each growing season is of vital interest in connection with the possible winter-injury of the trees due to desiccation during seasons of light rainfall.

The soil moisture present at the commencement of the dormant period of the trees is generally very small. Under the conditions which usually prevail, the last irrigation is applied late in August† just before the nuts ripen. The fall and early winter rains have come too early during the years 1919–1921, inclusive, to permit the study of the effect of a winter drought such as occurred in the two years before the irrigation experiment began. The studies here reported are therefore not conclusive proof of the effect of winter drought on walnut trees, but rather indicate the extreme dryness to which the soil is reduced at the commencement of the annual dormant period of the trees. When sufficient rain falls early in the dormant period the trees show no injury from autumn drought. Table 8 shows the moisture content of plots A, B, and C during the falls of 1919, 1920, and 1921.

It is apparent from this table and the discussion in a subsequent paragraph that the wilting point of the soil has no significance for the use of soil moisture by the walnut tree. The hygroscopic point is the critical point at which growth stops and the point at which the tree matures in a perfectly healthy and normal condition. Although this conclusion may not harmonize with many of the soil-moisture studies where annual crops have been used as indicators, it does agree with Alway's studies heretofore mentioned.

The continued dryness of the soil (at the hygroscopic point or below) throughout most of the root zone, is thus a matter of course

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\* The soil moisture content in the following discussion is stated as a ratio to the hygroscopic coefficient.

† The statement above applies to the irrigation practice in the majority of the walnut-growing sections. In the Hemet region most of the walnut groves are irrigated according to the schedule shown for plot B, on page 13.

during winter seasons of scanty rainfall. The same effect is produced in walnut groves in which is growing a winter barley crop which uses the moisture soon after it falls and thus prevents it from reaching the subsoil. Many observations made in barley fields during the winter of 1918-19 showed that 18 to 24 inches was the maximum depth which the rainfall reached, because, with the light occasional rains and the demands of the barley plants, there was no moisture left for deep penetration. During seasons of normal rainfall walnut trees are frequently severely injured by winter drought if intercropped with barley.

During the progress of this experiment a few cases of winter injury due apparently to drought have come to the attention of the authors. These have all occurred in intercropped orchards. Some have occurred in central California and it has been impossible to obtain any knowledge of the soil-moisture conditions which prevailed before the winter rains and the occurrence of the injury. These survey studies have still further strengthened the conclusions reported by the authors in 1919.<sup>1</sup>

Table 9 shows that in two young orchards, one of apricot and one of peach, on Placentia sandy loam, the latter underlain by impervious hardpan, the moisture content had been reduced below the hygroscopic point. The soil samples were taken December 1, 1921, 113 days after the last irrigation. A light shower occurred in October but it affected only the surface of the soil. The trees in these orchards were apparently entering a normal dormant period on December 1. The following spring (1922) these apricot and peach trees bloomed and started their normal spring growth, which would indicate that the olive and the walnut may not be exceptional in their ability to reduce the soil moisture to the hygroscopic point at the approach of their normal dormant period.

This condition may be considered common in fruit orchards in the semi-arid sections where the rains mainly occur during the dormant period. The reduction of the soil moisture to the hygroscopic point by tree roots apparently is not accompanied by a marked harmful effect, provided it is not prolonged too far into the winter period. It seems quite probable that the soil moisture between the wilting point and the hygroscopic point is of vital importance for maintenance of life and the ripening processes of tree growth.



FALL GROWTH OF WALNUT TREES IN RELATION TO DIFFERENCES IN  
SOIL MOISTURE

The length of the growing season of the walnut tree may be somewhat prolonged in the late summer and early fall by the presence of an abundance of soil moisture, and, vice versa, it may be shortened by a lack of sufficient soil moisture. The effect of late irrigation in prolonging the growth of the trees has been so often observed that it need not be dwelt upon at length in this paper; suffice it to say that it is often associated with injury to the trees by the first fall frosts, and for this reason alone the late irrigation of young walnut trees is inadvisable.

TABLE 9  
SOIL MOISTURE IN PEACH AND APRICOT ORCHARDS

Three-year-old peach orchard, December 1, 1921. (Trees nearly dormant, three-quarters defoliated.)

Ft.	Hygroscopic coefficient	Moisture observed	Ratio
1.....	4.91	2.98	0.61
2.....	5.42	4.81	0.89
3.....	6.16	5.84	0.95
Mean	—	—	0.82

Six-year-old apricot orchard, December 1, 1921. (Trees partly dormant, one-half defoliated, leaves yellowish green.)

Ft.	Hygroscopic coefficient	Moisture observed	Ratio
2.....	4.96	3.82	0.77
2.....	5.86	4.61	0.79
2.....	5.33	4.37	0.82
2.....	5.84	4.70	0.80
2.....	4.85	4.26	0.88
2.....	5.69	4.38	0.77
2.....	5.58	4.80	0.86
2.....	5.61	4.78	0.85
2.....	5.16	4.48	0.87
2.....	4.42	4.38	0.99
2.....	4.88	4.71	0.97
2.....	5.08	4.85	0.96
Mean			0.86

It has been of especial interest in this investigation to determine the dryness of the soil necessary to entirely check the growth of the tree and thus promote the commencement of the dormant period. The year 1921 was favorable for this sort of observation, inasmuch as the preceding winter rains were below the average and penetrated to a depth of only about 37 inches. With the addition of water from winter irrigation (see table 4) the depth of moist soil on plots A and C was not more than 6 feet. The water level in this grove is about 80 feet from the surface, and the soil below the reach of the rains or irrigation is constantly at a point of extreme dryness. Thus the lower root zone may remain at the hygroscopic point or slightly below this point for twelve to eighteen months at a time during seasons of scanty rainfall. Since summer irrigation did not penetrate more than 7 feet, it is reasonably certain that there was no soil moisture above the hygroscopic point in the lower root zone of the plots in question during the entire growing season of 1921. The greatest amount of water applied at any one time was 4.2 acre inches per acre, with twenty days as the shortest interval between irrigations.

Plots A and C were irrigated for the last time on August 9 and July 19, respectively. On September 13 it was noted that the trees on plot C were maturing somewhat faster than those on plot A. The foliage of the trees of plot C was beginning to turn yellowish green, especially the leaves in the centers of the trees, and a few leaves were beginning to collect on the ground. At the same time the foliage of the trees on plot A was still green. The twig growth had terminated on both groups of trees. None of the trees appeared wilted, although the maximum temperature at 2 P.M. was 85° F.; the relative humidity was 35 per cent, and the soil moisture was reduced to practically the hygroscopic point as shown in table 10.

The above observation was made 34 days after A was irrigated and 54 days after C was irrigated, both plots receiving 4.2 acre inches per acre. Since the same degree of dryness of the soil had been reached in the two plots, the difference in maturity of the trees may be taken to indicate that a prolonged period with the soil moisture between the wilting point and the hygroscopic point is necessary to check the growth of the walnut tree during the warm months of early fall.

The statement is made in a preceding paragraph that, "the soil below the reach of the rains or irrigation is constantly at a point of



extreme dryness.” It is of interest at this point to note observations which were made in this regard on November 17, 1922. A hole was bored 20 feet deep with a post hole auger, and each foot-level was kept separate for moisture observations. The following summary shows the relative moistness of the soil.

Foot-level	Hygroscopic point	Ratio of moisture observed to hygroscopic point
1	3.2	2.9
2	3.0	1.4
3	3.6	0.9
4	4.2	0.9
5	4.7	0.9
6	4.9	0.9
7	5.5	0.7
8	3.1	1.1
9	2.6	1.1.
10	4.3	0.9
11	2.8	0.8
12	1.7	1.5
13	2.6	1.2
14	1.3	1.1
15	5.4	1.1
16	6.8	1.0
17	4.2	1.3
18	5.9	0.9
19	4.8	0.9
20	4.4	0.9
Mean	4.0	1.0
(eliminating 1st foot)		

Except for the surface foot which had been wet by recent rains, and a sand layer in the twelfth foot, the moisture was very close to the hygroscopic point or below it.

TABLE 10

SUMMARY OF THE SOIL-MOISTURE OBSERVATIONS FOR PLOTS A AND C, SEPTEMBER 13, 1921, EXPRESSED AS A RATIO OF THE HYGROSCOPIC POINT

	Plot A	Plot C
Average of 1st ft.....	0.91	0.72
Average of 2nd ft.....	0.98	0.91
Average of 3rd ft.....	0.98	1.00
Average of 4th ft.....	0.93	1.14
Average of 5th ft.....	1.02	0.91
Average of 6th ft.....	1.01	1.05
Average of 7th ft.....	1.06	1.11
Mean	0.98	0.98

## SUMMARY

1. The rainfall of the Hemet Valley is less effective in raising the soil-moisture content of the subsoil than might be thought from a mere statement of total rainfall. Much of the rain falls in small showers of only a few tenths of an inch and is rapidly lost by surface evaporation. For a three-year period the total depth of penetration of moisture averaged 5.2 inches per inch of rainfall during the rainy season.

2. Studies made upon the moisture content of the soil in a walnut grove have shown that at the end of the growing season the moisture was reduced to a point near the hygroscopic coefficient, in spite of summer irrigations totaling 12.5 acre inches for the season. After a period of 169 days without a rain of .3 of an inch or more the moisture in the upper five feet of this soil varied from .54 to .85 of the hygroscopic coefficient. The moisture content of a different type of soil in a three-year-old peach orchard and in a six-year-old apricot orchard showed a similar degree of dryness at the same state in the growth cycle of the trees. The average moisture content in the upper three feet of the former soil was .82 of the hygroscopic coefficient, and in the second foot of the latter was .86 of the hygroscopic coefficient.

3. In spite of the low moisture content of the soil in the latter part of the growing season, the trees showed no permanent wilting but continued to mature and entered the dormant period without apparent injury. Temporary wilting occurred only during the middle of the day when a high temperature was accompanied by a low humidity.

4. The moisture content of the soil was generally at the hygroscopic point at the end of the growing season whatever the amount of water present at the beginning. In other words there was no residuum of water for the use of the trees in the following season.

5. The moisture content of the upper seven feet of the soil in this grove gradually increased with the winter rainfall and usually reached a maximum percentage in March, when the moisture present equaled from 2.5 to 3.5 times the hygroscopic coefficient. Early in April the amount of soil moisture was reduced as the trees started their spring growth. The soil moisture of the upper four feet was approximately at the wilting point by the middle of June. The residual moisture from heavy winter irrigation persisted in the subsoil area until the



middle of the growing season, but by the end it had been gradually taken out of this area, where the tree roots are less numerous. Summer irrigations of 4.2 acre inches per acre raised the water content for a brief time, but within thirty days it dropped back to the approximate value of the wilting point. Such irrigation had little effect on the water content of the soil below the fourth foot. By the end of October the soil in the upper seven feet was approximately at the hygroscopic point.

6. In stating the moisture content of the soil in relation to any of the various conventional coefficients, such as the moisture equivalent, the wilting point, or the hygroscopic coefficient, the field studies with walnuts have shown that the hygroscopic coefficient is the most logical point upon which to base all calculations. If the actual moisture present is stated in comparison with the wilting point, for example, we shall be dealing with minus quantities much of the time. It has seemed to the authors that statement of the moisture in a soil as a ratio of the hygroscopic coefficient gives the most comprehensive conception of the relative moistness. When such a ratio is accompanied by a statement of the hygroscopic coefficient, the type of soil worked with can be clearly understood by the reader.

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THE EFFECT OF THE PLANT ON THE  
REACTION OF THE CULTURE  
SOLUTION

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For several years considerable attention has been given in this laboratory to the study of the absorption of ions by various plants under controlled solution culture conditions. Incidental to this work, numerous observations have been made of the changes in the reaction of culture solutions induced by the growth of various plants, and it is now intended to discuss very briefly certain data relating to this phase of the investigation, and to suggest the bearing which the experiments in question may have on the processes associated with the intake of ions by plants. While the literature pertaining to the relations between hydrogen ion concentration and biological phenomena has now reached enormous proportions, the extension of knowledge in this field is still profitable, since the reaction of the medium may frequently become the most important variable in biological systems, as has been so clearly illustrated, for example, by Loeb<sup>1</sup> in his recent enlightening researches on proteins and colloidal behavior.

In a previous paper<sup>2</sup> attention was called to the fact that ordinary culture solutions containing nitrate, when placed in contact with growing plants, tend to change their reactions very rapidly. If the initial reaction be acid, the pH value of the solution increases

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\*The writer desires to acknowledge the assistance of Mr. D. C. Caudron in certain of the experiments.

and generally attains an approximately constant value close to the neutral point. Subsequent experiments in different laboratories have, on the whole, been in agreement with this finding.<sup>3, 4</sup> While different solutions show a similar tendency in this regard, the rate at which the reaction is altered is necessarily retarded in a solution like Shive's, which possesses a relatively high buffer value associated with its high proportion of phosphate. This has been shown by Jones and Shive<sup>3</sup> in their comparisons of different culture solutions.

Since the concentration of hydrogen ions undergoes a definite change, obviously there must take place a readjustment in the concentrations of certain other ions. The nature of these changes is illustrated by some determinations of the quantities of the different ions removed from culture solutions by barley plants at different stages of growth. Solutions of similar composition were compared, one of an initial pH value of 5.1, the other 6.8. The solutions were changed weekly and the absorption of ions determined for three separate periods of one week each, beginning with the fourth, sixth and eleventh weeks of growth (table 1).

In this experiment, the total equivalents of anions removed from the acid solution very considerably exceeded those of cations. The equivalence of positive and negative ions was maintained in the solution by the formation of  $\text{HCO}_3$  ions. Thus an ion not originally present in appreciable concentration soon appeared in the culture solution in significant quantity as a result of the activities of the plant. This replacement of the anion of a strong acid (chiefly  $\text{NO}_3$ ) by the weak acid anion  $\text{HCO}_3$ , seems to determine in large measure the change of reaction in culture solutions. Such solutions, however, do not ordinarily attain an alkaline reaction (unless the solution is allowed to concentrate too far), since the excretion of carbon dioxide by the plant prevents the formation of normal carbonate. Under these conditions, the approximately constant reaction reached is very similar for quite diverse types of plants. Theron<sup>5</sup> has determined, for example, the following values: Peas, pH 6.7, barley and corn pH 6.8.

In the experiments referred to above, it may also be noted that the absorption of Ca and  $\text{PO}_4$  ions was greater from the more acid solution. The sum of the equivalents of positive and negative ions removed was greater from the solution of pH 5.1 than from the solution of pH 6.8. In two periods of growth, approximately equivalent



quantities of anions and cations were removed from the solution having an initial pH value of 6.8.

It may be asked whether the reaction to which the solution is brought by the growth of the plant is, therefore, the most favorable one for growth. No doubt excellent development of many plants is possible at this reaction, but it cannot be asserted that it is necessarily the optimum. With certain plants, for example, barley, cucumbers, peas, an appreciably more acid reaction, continuously maintained in this solution, seems to be accompanied by increased plant growth, at least in the early stages. It should be added that in no case which has come under our observation has a solution with a pH value greater than 7 produced yields of plant as great as those obtained from slightly acid solutions. In a paper just published, Bryan<sup>6</sup> concludes that alfalfa and clover produce maximum growth at pH 7 and pH 8. In these investigations, however, it is possible that the pH values of the alkaline solutions (quartz sand cultures were used) decreased very rapidly, especially in the zones immediately in contact with the absorbing root membranes, as a result of selective absorption and high concentration of CO<sub>2</sub>. Our own results with alfalfa in solution culture indicate that pH 8 is less favorable to growth than a slightly acid reaction. It does not follow, of course, that plants do not exist which can grow well in an alkaline medium. In fact, we have found that Bermuda grass thrives even at pH 9. This statement would also apply, no doubt, to numerous other alkali tolerant plants.

Another question which may be raised in this connection is concerned with the necessity of providing in the soil or culture solution a supply of carbonates or bicarbonates. It has been stated, for example, that calcium must be present in the form of carbonates or bicarbonates in order to effect the required neutralization of acid developed within the plant as a by-product of the nitrogen or carbohydrate metabolism. In the culture solutions referred to in this paper, no carbonates or bicarbonates were included, and yet all the species of plants tested have made excellent growth. It is true that bicarbonates are soon formed in solutions of this type, but this is a result of the plant's own metabolism. Furthermore, certain plants, including several legumes, have been grown in solutions continuously maintained at pH values of 4.5–5.0, by the frequent addition of suitable amounts of acid without any ill effects on the plant. In such



solutions only very small amounts of bicarbonate ion are present. For these reasons, it is difficult to reach the conclusion that bicarbonates are in general essential for the growth of many common agricultural plants, at least when nitrogen is supplied in the form of nitrate. Certainly, the plants which we have experimented with can easily effect the formation of bicarbonate in such solutions. No doubt, small quantities of bicarbonate ion may be present in the juices of many plants, as suggested by Haas,<sup>7</sup> but the metabolism of the plant could account for this condition, irrespective of external sources of bicarbonate ion. When nitrogen is supplied to the plant in the form of ammonium salts, sulphate or chloride, the culture solution may reach an acidity of pH 3.2, according to certain experiments on barley plants carried on in this laboratory. This observation is in general agreement with Olsen's<sup>8</sup> recent results on other plants. The presence of a suspension of calcium carbonate prevents the solution from reaching this injurious acidity, but it is not essential to relate the improved environment to the presence of calcium bicarbonate as such, since other basic ions are also in equilibrium with bicarbonate ion, and take part in the buffer system of the plant. Calcium, of course, may possibly play a special role in some plants by virtue of its ability to form insoluble salts with certain organic acids. To what extent such precipitations occur in the common agricultural plants remains to be shown, but, in any event, it does not appear to be justifiable to assign to calcium an exclusive role in the regulation of the reaction of plant juices. Potassium, for example, in equilibrium with organic acid anions, must possess a buffer effect of very important magnitude. As a matter of fact, such plants as barley and peas may show a slightly decreased acidity in their juices when the supply of calcium in the culture solution is restricted, as evidenced by a number of experiments made in this laboratory by Newton.<sup>11</sup> Haas<sup>7</sup> also cites several similar cases based on soil studies.

In conducting experiments with alkaline culture solutions, several serious difficulties are confronted. In the first place, certain of the essential constituents, notably calcium, iron and phosphate, may precipitate out. It is also not a simple matter to maintain a given alkalinity in the solution, because of the marked tendency of the plant to decrease the OH ion concentration. It is not ordinarily feasible to use a solution of sufficiently high buffer value which

is otherwise adapted to plant growth. Possibly the best method would involve the principle of a continuously flowing solution, but such an arrangement is not always practicable. In this laboratory, Theron<sup>5</sup> carried on a series of experiments in which very large volumes of solutions were employed and alkali or acid added frequently (twice daily when necessary) so as to maintain with fair constancy the desired reaction. These data will be reported elsewhere in more detail, but it is desirable to state here that a greater proportion of cations was removed from the alkaline solutions than from those with an acid reaction. The decrease of alkalinity was the result of this selective absorption of bases and also of the excretion of carbon dioxide from the plant roots. The decrease of alkalinity in various single salt solutions is made manifest by certain of the data cited in this paper. It may be well to emphasize again the fact that when sand cultures are employed, the maintenance of a constant alkaline reaction in a solution in immediate contact with roots is almost impossible when solutions of suitable concentration are used. This fact must be taken into account in the interpretation of sand culture experiments.

The solutions so far under discussion have been complete culture solutions and are, therefore, of extremely complex nature. It is frequently interesting and enlightening to experiment with single salt solutions (compare Gericke<sup>10</sup>). In some preliminary tests made a number of years ago, the effect of the plant on the reaction of several such solutions was observed, and it was concluded that no injurious intensity of acidity or alkalinity was developed. In these experiments, the plants were grown for six or seven weeks in complete culture solutions and were then transferred to the solutions of single salts, after rinsing the roots of the plants in distilled water. In continuance of this work, plants have been transferred to the single salt solutions immediately after germination, or after only a limited period of contact with the complete culture solution. Under these circumstances more extensive changes of reaction have occurred in various salt solutions. The plants were grown in vessels of 120 c.c. capacity, from thirty to fifty plants generally being used for each solution. The cultures were carried on at various times of the year, in most instances outdoors, but in several experiments the plants were placed in a heated greenhouse. The reactions were determined on composite samples by standard colorimetric methods. The buffer



effect of the solutions were frequently very slight, and in such cases the values obtained can be regarded as only approximate. Definite changes of reaction produced by the plant can be demonstrated, however, without difficulty. The total concentrations of the solutions were of magnitudes found in many culture and soil solutions.

Three types of plants were used in the present experiments: Barley (Beldi and Mariout variety), peas (Dwarf Wonder variety), cucumbers (White Spine variety).

The measurements of the reactions of the different solutions, after various periods of contact with different plants, are given in tables 1 to 4. These data show that solutions of certain salts were consistently changed to a reaction more acid than the initial one, as a result of even relatively brief contact with the roots of the plants whether of barley, peas or cucumbers (tables 2, 3, 4). The ammonium salts are particularly subject to an increase of hydrogen ion concentration. The other solutions in which a significant decrease of pH value was generally brought about include  $K_2SO_4$ ,  $Na_2SO_4$ ,  $KH_2SO_4$ . Peas, barley, and cucumbers all exhibited this tendency. Breazeale<sup>11</sup> by titration methods, showed that an increased acidity was produced in  $K_2SO_4$  solutions by the growth of wheat seedlings. A very interesting instance of increase of acidity was furnished by cucumber plants growing in solutions of calcium salts. In a number of cases, a very definite decrease of pH value took place. This effect was also found in one experiment with peas grown in similar solutions. It does not follow, however, that peas will absorb more Ca than barley from a complete culture solution. This was not found to be the case in the experiments of Newton.<sup>9</sup>

The solutions of the following salts tended to have their hydrogen ion concentrations decreased when barley plants were grown in them:  $(Ca(NO_3)_2)$ ,  $CaCl_2$ ,  $Mg(NO_3)_2$ ,  $NaNO_3$ ,  $Ca(H_2PO_4)_2$ ,  $Mg(H_2PO_4)_2$ ,  $NaH_2PO_4$ . In the case of any nitrate (except ammonium), the solution generally attains a reaction very close to neutrality, but the precise reaction will be determined by the quantity of free  $CO_2$  present. Under favorable weather conditions the growth of peas and cucumbers caused an increase of acidity in  $CaSO_4$  solutions to pH 4.0.

It is important to note that the changes of reaction of certain salt solutions are not always the same in the different experiments. Obviously the rate of growth and of ion absorption will determine



the rate at which a given solution can undergo a modification of hydrogen ion concentration, so that the factors of light and temperature are necessarily involved. For example, it was noted in one experiment that even a very slight shading of barley cultures markedly diminished the rate of increase of acidity in a  $K_2SO_4$  solution. After a short period of growth, the cultures nearest the window had a pH value of 4.0, and those at the opposite end of the row a pH value of 5.7. It would appear in a few cases that not only the rate but the direction of the change of reaction may be altered, according to the rate of growth. In the case of sodium and potassium sulphates, of various nitrates, and of ammonium salts, the effects are, in general, consistent for the different plants and under varying conditions of growth.

When non-buffered salts are used and at reactions where carbon dioxide has an important influence, it is not justifiable to place any emphasis on small fluctuations of hydrogen ion concentration, since this will vary with the rate at which carbon dioxide is given off by the roots and also with the temperature of the solution. The solutions having the higher acidities (pH 4.0 and less) were not influenced by this factor, since the reaction was not changed by boiling.

The effect of the plant on the reaction of the culture medium is of interest to any consideration of the absorption of ions by the plant. One of the most striking facts connected with this absorption is the ease with which  $NO_3$  ions are removed from solution and replaced by  $HCO_3$  ions. One can only speculate concerning the mechanism involved. The effect would be the same whether H ions and  $NO_3$  ions were removed, leaving the cations and  $HCO_3$  ions, formed as a result of  $CO_2$  excretion by the plant, or whether there were some sort of exchange of  $HCO_3$  and  $NO_3$  ions. This replacement of an anion by  $HCO_3$  ion is not necessarily limited to the nitrate ion. When actively growing barley plants (previously in contact with a complete culture solution) were placed in a solution of  $CaCl_2$ , more chlorine than calcium was absorbed and very appreciable amounts of  $HCO_3$  ion were formed. Rudolfs<sup>12</sup> has recently investigated the effects of contact of different solutions with the seeds of different plants. In general, there was tendency for a slightly increased but definite acidity to develop in all solutions. The change of reaction in nitrate solutions was, therefore, different from that produced by growing plants.

From a solution of  $K_2SO_4$ , either K and OH ions (or  $HCO_3$  ions) are removed, or else K ion is exchanged for hydrogen ion. The solution being initially acid from the presence of carbonic acid, the concentration of OH ions must necessarily be very small, which would presumably operate to restrict the absorption of K ion, accompanied by OH ion.

Particular interest attaches to the absorption of ammonium ions. This ion seems to penetrate into the living cell with the greatest ease. Jacobs<sup>13</sup> has shown this by direct observations on certain plant and animal cells, and Davis and the writer<sup>14</sup> have likewise found that the reaction of the vacuolar sap of *Nitella* (a fresh water alga producing large cells from which practically uncontaminated sap may be obtained) can readily be changed when the cells are immersed in even very dilute solutions of ammonium salts. The consequence is that the cell is quickly injured. As already stated, an increase of acidity was readily brought about in solutions of ammonium salts by the growth of barley, peas or cucumbers.

If one ion is absorbed more rapidly than the accompanying oppositely charged ion, it might be thought that the reaction of the root juices would also undergo change as well as that of the solution. In some of the experiments, determinations were made of the approximate hydrogen ion concentrations of the juices expressed from roots grown in solutions of various salts. There were some marked variations, but, in general, it was not possible directly to relate the change of reaction to the effect of the ion absorbed in excess. For example, the absorption of ammonium ion did not increase the alkalinity of the root juices, as far as could be ascertained. Probably this increase should not be expected in view of the influence of the buffer system of the plant, the immediate translocation of substances away from the root and the rapid metabolism of nitrogen compounds. After the elapse of sufficient time, however, the acidity induced in certain solutions ( $KH_2SO_4$ ,  $(NH_4)_2SO_4$ , and others), caused the reaction of the root juices to become more acid. In such cases, the roots were always definitely injured. With peas, it was noted that injury and discoloration affected the roots of the plants grown in solutions of  $KNO_3$  and  $NaNO_3$ , but not of  $Ca(NO_3)_2$ . The juices from these injured roots exhibited a slightly higher alkalinity than normal. The hypothesis may be advanced that an alkaline residue was formed as a result of the rapid reduction or translocation of  $NO_3$  ion.



It may be of interest to add several additional comments on the effects of various salts on root development. With barley, better development was obtained in a potassium nitrate solution than in a calcium nitrate solution. All calcium salts permitted excellent development of the roots of peas and cucumbers. In both cases, solutions containing calcium carbonate in suspension offered a very favorable medium for root growth, but it was not appreciably superior to a solution of calcium sulphate. It should be stated that the solutions containing an excess of calcium carbonate may, nevertheless, have a slightly acid reaction, because of the carbon dioxide given off by the plant roots. Cucumbers were extremely sensitive to sodium salts, so much so that any determinations of changes of reaction in such solutions are not of much value. With all three plants, injury was pronounced in those solutions which attained an acidity represented by a pH value of less than 4.

#### DISCUSSION

The data presented in this paper justify the conclusions previously reached with regard to complete or partial culture solutions containing nitrate, namely that there is a very general tendency for the plant to change the reaction toward the neutral point, whether the initial reaction be acid or alkaline. The results obtained with certain single salts make it necessary to modify the previous conclusion that the plant does not cause a solution to change its reaction to a point injuriously acid. This did not occur, in fact, in earlier experiments, with plants grown for some time in complete culture solutions and which continued to grow in the single salt solutions without injury, but when seedlings are transferred directly to the solutions (or after only a limited contact with the complete culture solution) of such salts as  $(\text{NH}_4)_2\text{SO}_4$  or  $\text{K}_2\text{SO}_4$ , certainly the acidity developed was detrimental to root growth, and eventually highly injurious to the plant as a whole.

The actual excess absorption of one ion is necessarily very slight. Acidities greater than pH 3.0 or 3.2 were not observed to develop, and this concentration of hydrogen ion would correspond to only an extremely small selective removal (or exchange) of cations. It is true that frequently a very substantial excess of cation is actually removed from the solution as shown by chemical analysis, but it has



been shown that in these solutions much of the discrepancy can be accounted for by the loss from the plant of some other cation. In other words, a sort of exchange of bases takes place. (Whether or not this occurs in the cell wall remains to be demonstrated.) Furthermore, a slowly diffusing anion, especially sulphate ion, retards very materially the absorption of the associated cation. In this connection, attention is directed to the fact that these properties of the ions are not to be explained on the simple assumption that certain ions are required by the plant and are, therefore, "selected" out of the solution. The increase of acidity may be produced in sodium sulphate, as well as in potassium sulphate solutions. Chlorine ion is removed from solution, by some plants at least, much more readily than sulphate ion. Many experiments have been carried out bearing on these statements but the results can be presented more conveniently in a later discussion.

The absorption of ions by a plant cell is by no means a matter of simple chemical equilibrium. The general nature of the problems can be most clearly illustrated by a reference to some recent experiments on *Nitella*.<sup>14 15 16 17</sup> This plant produces cells sufficiently large so that sap may be obtained from individual cells practically uncontaminated. The investigations with *Nitella* prove conclusively that the major portions of the inorganic elements contained in this plant exist as dissociated compounds. Ions are absorbed into the cell sap from an outside solution of much lower concentration. In other words, the plant must apparently do work in connection with this absorption. Possibly, however, the first step in the process is the formation of some sort of an ion compound, and this may be dependent, at least in the case of some ions, on the hydrogen ion concentration of the solution. It was found that nitrate penetrated far more rapidly into the cell sap of *Nitella* from an acid solution than from an alkaline solution. These remarks are included in this discussion because it is desired to emphasize the dynamic nature of the processes of ion absorption and of the change of reaction induced in solutions by the plant. It is also of interest to suggest the importance of Loeb's work on the chemical nature of proteins in this general connection. The maintenance of a suitable reaction within the cell is probably of paramount importance, because the hydrogen ion concentration may be one of the chief variables governing the colloidal behavior of the protoplasm. In addition to this, if any intermediate

combinations of protein and ions should be concerned in the mechanism of absorption, these must be intimately related to the hydrogen ion concentration of the solution.

Briefly referring to the soil phase of the question, it is doubtful whether the activities of the plant in the absorption of ions ordinarily bring about directly an increase of acidity in the soil. The principal source of nitrogen is generally in the form of nitrate and this ion will normally be absorbed with such rapidity that bicarbonate residues are left. Observations on soil extracts and displaced solutions give evidence that during normal crop growth and at the time of maximum absorption, practically every trace of nitrate is removed from the soil solution. The cations are also reduced in concentration to a greater or less degree but certainly not to so great an extent as the nitrate ion. Therefore, it would seem necessary to conclude that bicarbonate ion is correspondingly increased in concentration, just as it is in solution cultures. The continued use of ammonium salts on a soil generally causes, in course of time, an increased hydrogen ion concentration, but here it is essential to consider also the activities of micro-organisms, which is not within the scope of this paper.

#### SUMMARY

1. The reaction of a culture solution has an important bearing on the absorption of ions by plants. The absorption of  $\text{NO}_3$  ion was found to be favored by an acid reaction, and a relatively increased absorption of cations occurred when an alkaline reaction was present.

2. Observations were made of the effect produced on the reaction of various single salt solution by the growth of barley, peas, and cucumbers. An increase of acidity occurred with a number of salts, particularly ammonium sulphate and chloride, potassium or sodium sulphates, and potassium phosphate. Injury to the plant was produced by the acidity developed.

3. The importance of light and temperature as influencing the absorption of ions and change of reaction in culture solutions, is emphasized.

4. The necessity for considering the part taken by  $\text{CO}_2$  and by  $\text{HCO}_3$  ion in any explanation of the effect of the plant on the reaction of culture solutions is pointed out.



5.  $\text{KNO}_3$  in single salt solution caused injury to the roots of pea plants, but not to those of barley.

6. The general question of the relation between ion absorption and hydrogen ion concentration is briefly discussed. Attention is called to the possible bearing of Loeb's work on proteins in this connection.

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TABLE 1

ABSORPTIONS OF IONS BY BARLEY PLANTS FROM ACID AND NEUTRAL SOLUTIONS

(Computed in milli-equivalents, absorbed by each culture during periods of one week)

Period of growth	Initial pH value	K	Ca	Mg	NO <sub>3</sub>	PO <sub>4</sub>	SO <sub>4</sub>	Total positive ions	Total negative ions	Differ- ence*	Sum of positive and negative ions
4-5 weeks.....	5.1	2.30	2.40	.66	6.18	.63	.87	5.36	7.68	-2.32	13.04
	6.8	1.66	1.40	.74	2.53	.45	.48	3.80	3.46	+ .34	7.26
6-7 weeks.....	5.1	3.23	4.59	1.64	8.62	1.13	1.48	9.46	11.23	-1.77	20.69
	6.8	3.20	3.04	1.32	6.70	.64	1.35	7.56	8.69	-1.13	16.25
11-12 weeks.....	5.1	2.74	4.19	1.40	8.73	1.53	1.50	8.33	11.81	-3.48	20.14
	6.8	2.87	3.69	1.89	5.23	.98	1.68	8.45	7.89	+ .56	16.34
Composition of Original Solution.....	5.1	6.35	14.80	7.40	17.70	1.89	7.16	(Small quantities bicarbonate and silicate ions also present)			
	6.8	6.45	14.30	8.38	16.10	1.39	8.22				

20 cultures and 40 plants used for each solution

\* Referable to undetermined bicarbonate ion chiefly, not to errors in analysis.

TABLE 2  
EFFECT OF PLANT ON REACTION OF SOLUTION

Experiment 1—(Barley)										Experiment 2—(Barley)									
Salt		Concentra- tion, Milli-equiv.	pH Values					Salt	Concentra- tion, Milli-equiv.	pH Values									
			Initial	After 2 days	After 7 days	After 11 days	After 17 days			Initial	After 2 days								
KNO <sub>3</sub> .....	6.40	6.5	.....	5.7	5.7	5.7	6.0	Mg(NO <sub>3</sub> ) <sub>2</sub> .....	20.8	5.0	6.7								
KCl.....	6.40	6.0	6.8	5.5	5.5	5.5	5.5	MgSO <sub>4</sub> .....	20.8	5.0	5.3								
K <sub>2</sub> SO <sub>4</sub> .....	6.40	6.0	3.3	3.4	3.4	3.1	3.4	Mg(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> .....	20.8	4.0	4.7								
KH <sub>2</sub> PO <sub>4</sub> .....	6.40	5.1	3.3	3.4	3.4	3.8	3.7	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	12.5	5.6	7.0								
KHCO <sub>3</sub> .....	6.40	8.4	8.4	6.0	6.0	5.7	6.0	CaCl <sub>2</sub> .....	12.5	5.8	6.6								
NaCl.....	10.75	5.7	7.2	6.1	6.1	7.2	7.0	CaSO <sub>4</sub> .....	12.5	6.4	6.6								
Na <sub>2</sub> SO <sub>4</sub> .....	10.75	4.9	3.8	3.4	3.4	3.4	3.4	Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> .....	12.5	4.2	4.2								
NaHCO <sub>3</sub> .....	10.75	8.4	8.4	7.2	7.4	7.4	7.2												
Plants grown 26 days in complete culture solution before placing in single salt solutions										Plants grown 22 days in complete culture solution									
Experiment 3—(Barley)										Experiment 5—(Barley)									
Salt		Concentra- tion, Milli-equiv.	pH Values		Salt	Concentra- tion, Milli-equiv.	pH Values		Salt	Concentra- tion, Milli-equiv.	pH Values								
			Initial	After 2 days			Initial	After 5 days			Initial	After 14 hours							
Mg(NO <sub>3</sub> ) <sub>2</sub> .....	4.00	5.4	7.2	7.2	K <sub>3</sub> PO <sub>4</sub> .....	6.40	9.2	7.2	KNO <sub>3</sub> .....	1.97	5.0	6.8							
KNO <sub>3</sub> .....	4.00	6.0	7.2	7.2	KH <sub>2</sub> PO <sub>4</sub> .....	6.40	7.4	7.0	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	.20	5.0	6.8							
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	4.00	6.8	7.2	7.2	K <sub>2</sub> CO <sub>3</sub> .....	6.40	9.9+	7.3	KNO <sub>3</sub> .....	5.90	5.0	6.8							
NaNO <sub>3</sub> .....	4.00	6.6	7.0	7.0	KHCO <sub>3</sub> .....	6.40	9.9+	7.4	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	.57	5.0	6.8							
Mg(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> .....	2.50	4.0	5.4	5.4	K <sub>2</sub> SiO <sub>3</sub> .....	6.40	9.2	7.3	KNO <sub>3</sub> .....	9.90	5.0	6.8							
KH <sub>2</sub> PO <sub>4</sub> .....	2.50	4.7	5.3	5.3					Ca(NO <sub>3</sub> ) <sub>2</sub> .....	.94	5.0	6.8							
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> .....	2.50	3.7	5.6	5.6	Plants grown 40 days in complete culture solution					KNO <sub>3</sub> .....	14.80	5.0	6.8						
NaH <sub>2</sub> PO <sub>4</sub> .....	2.50	4.7	5.4	5.4						Ca(NO <sub>3</sub> ) <sub>2</sub> .....	1.42	5.0	6.8	KNO <sub>3</sub> .....	19.90	5.0	6.8		
Plants grown 19 days in complete culture solution										Ca(NO <sub>3</sub> ) <sub>2</sub> .....	1.88	5.0	6.8						
										KNO <sub>3</sub> .....	29.90	5.0	6.8						
										Ca(NO <sub>3</sub> ) <sub>2</sub> .....	2.82	5.0	6.8						
										KNO <sub>3</sub> .....	39.60	5.0	6.8						
										Ca(NO <sub>3</sub> ) <sub>2</sub> .....	3.76	5.0	6.8						
										Plants grown 19 days in complete culture solution									



TABLE 3

EFFECT OF PLANT ON REACTION OF SOLUTION

Salt	Concen- tration, Milli- equiv.	pH Initial	EXPERIMENT 1* (Peas)					EXPERIMENT 2 (Cucumbers)			EXPERIMENT 3 (Barley)	
			pH Values					pH Values			pH Values	
			After 3 days	After 6 days	After 13 days	After 17 days	After 21 days	After 5 days	After 9 days	After 13 days	After 7 days	After 11 days
KNO <sub>3</sub> .....	10.0	5.0	5.4	5.0	5.4	6.2	6.4	5.6	5.0	5.2	5.4	6.6
KCL.....	10.0	5.2	4.8	4.8	4.8	5.0	6.0	5.2	4.2	5.0	4.8	4.0
K <sub>2</sub> SO <sub>4</sub> .....	10.0	5.2	5.0	4.8	4.4	4.4	4.4	5.0	4.4	5.0	3.8	3.4
KH <sub>2</sub> PO <sub>4</sub> .....	10.0	4.6	4.8	4.6	4.4	4.2	4.2	4.6	4.6	4.6	3.6	3.6
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	10.0	5.0	5.6	5.8	5.8	6.8	6.6	5.8	5.6	4.6	6.8	6.8
CaCl <sub>2</sub> .....	10.0	5.2	5.0	5.0	5.0	5.2	5.6	5.2	5.2	5.0	5.2	5.4
CaSO <sub>4</sub> .....	10.0	6.0	5.2	6.0	5.4	5.8	5.2	5.2	4.6	4.0	5.6	4.8
NaNO <sub>3</sub> .....	10.0	4.6	5.4	5.2	5.4	5.8	6.0	5.8	5.8	6.4	5.6	6.2
NaCl.....	10.0	5.0	5.0	4.8	5.0	5.4	5.0	5.8	5.6	5.8	4.2	4.0
Na <sub>2</sub> SO <sub>4</sub> .....	10.0	5.2	5.0	5.2	5.0	5.6	6.0	5.8	5.0	5.2	3.8	3.6
NH <sub>4</sub> NO <sub>3</sub> .....	10.0	5.0	5.2	5.0	5.0	5.2	5.4	5.2	5.0	4.4	4.0	5.0
NH <sub>4</sub> Cl.....	10.0	5.2	4.8	4.6	5.0	5.0	5.0	5.0	4.8	4.0	3.4	3.2
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	10.0	5.0	5.2	4.8	5.0	5.0	.....	5.0	4.8	4.0	3.4	3.2

Seedlings placed in single salt solutions immediately after germination

\*Unfavorable weather conditions

TABLE 4  
EFFECT OF PLANT ON REACTION OF SOLUTION

EXPERIMENT 1—(Peas)					EXPERIMENT 2—(Peas)					
Salt	Concen- tration, Milli- equiv.	pH Values			Salt	Concen- tration, Milli- equiv.	pH Values			
		Initial	After 4 days	After 12 days			After 19 days	Initial	After 7 days	After 15 days
NH <sub>4</sub> NO <sub>3</sub> .....	10.0	5.8	4.4	4.3	KNO <sub>3</sub> .....	10.0	5.8	5.2	5.6	6.6
NH <sub>4</sub> CL.....	10.0	5.8	4.0	3.9	K <sub>2</sub> SO <sub>4</sub> .....	10.0	5.8	4.6	4.0	3.9
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	10.0	5.8	4.2	4.2	Na <sub>2</sub> SO <sub>4</sub> .....	10.0	6.0	4.6	4.4	4.3
					CaCl <sub>2</sub> .....	10.0	5.8	5.2	5.0	4.2
					CaSO <sub>4</sub> .....	10.0	5.8	5.4	5.1	3.9
					NH <sub>4</sub> NO <sub>3</sub> .....	10.0	5.8	4.8	4.9	5.1
					NH <sub>4</sub> Cl.....	10.0	5.8	4.3	4.1	4.2
					(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	10.0	5.8	4.3	4.1	4.1

Seedlings placed in single salt solutions immediately after germination

Seedlings placed in single salt solutions immediately after germination

EXPERIMENT 3—(Cucumbers)					EXPERIMENT 4—(Cucumbers)				
Salt	Concentra- tion, Milli-equiv.	pH Values			Salt	Concentra- tion, Milli-equiv.	pH Values		
		Initial	After 9 days	After 17 days			Initial	After 6 days	After 14 days
KNO <sub>3</sub> .....	10.0	5.1	5.5	5.8	KNO <sub>3</sub> .....	10.0	5.2	6.1	.....
KCl.....	10.0	4.9	4.5	4.3	K <sub>2</sub> SO <sub>4</sub> .....	10.0	5.2	4.2	3.9
K <sub>2</sub> SO <sub>4</sub> .....	10.0	5.0	4.2	4.3	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	10.0	5.4	6.5	6.8
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	10.0	5.0	5.8	5.6	CaCl <sub>2</sub> .....	10.0	5.4	5.8	6.0
CaCl <sub>2</sub> .....	10.0	5.0	5.5	5.0	CaSO <sub>4</sub> .....	10.0	5.4	5.2	4.2
CaSO <sub>4</sub> .....	10.0	5.6	5.6	5.0	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	10.0	5.2	5.1	5.1

Seedlings placed in single salt solutions immediately after germination

Unfavorable growing conditions  
Seedlings placed in single salt solutions immediately after germination



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SOME MUTUAL EFFECTS ON SOIL AND PLANT  
INDUCED BY ADDED SOLUTES

BY

JOHN S. BURD AND J. C. MARTIN

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A recent paper<sup>5</sup> dealing with the effects of salts on soils states that "the solid, liquid and gaseous phases of a soil form a chemical system and a material change in any one of these phases must inevitably affect the equilibrium of the system." Application to the soil of fertilizers, irrigation waters carrying soluble salts, or of any substance capable of dissolving in water will obviously affect the liquid phase and hence modify the soil's equilibrium. A most important consequence of the recognition of the fact that the soil comprises a dynamic system is that the investigator may no longer hope to correlate plant production directly with the amounts of the specific chemical elements or of salts applied to the soil. Such correlations as are developed must be between production and increases or decreases in the amounts of the various solutes in the effective solution of the soil.

Working with 0.01 N. solutions in the proportion of five parts of solution to one of soil, Kelley and Cummins<sup>5</sup> have shown that, after one hour of contact:

"1. Chemically equivalent solutions of the chlorides, sulfates and nitrates of a given base, produced substantially equivalent chemical reactions in the soils studied.

"2. The solubility of the anion of the neutral solutions was not materially affected by the soils studied, but an exchange of bases took place, with the result that a portion of the base of the added salt passed out of solution and a chemically equivalent amount of other bases was set free from the soil silicates."

In the experiments upon which these conclusions were based, there was no change in reaction in any of the soils, and, as stated, the anions were not materially affected. The evidence is fairly convincing that, under such circumstances, the interaction of salts with soils is in general a strictly stoichiometric one.

In this laboratory, working with several soils (the same soils discussed hereinafter with reference to other experiments), one of us has shown that when the soils contained large quantities of solutes and were mixed with small amounts of potassium chlorid, the potassium fixed was equivalent to the amounts of calcium, magnesium, and sodium brought into solution. As the proportion of potassium chlorid to soil was increased, the agreement between the reaction values of potassium removed and those of other bases entering the solution was fair, but not in all cases conclusive of an exact equivalence. Using the same soils after very thorough leaching and with the same proportions of potassium chlorid to soil as in the former experiment, the agreement between the reaction value of potassium fixed and other bases rendered soluble was good in the case of only one soil, and then only when the smallest proportion of added potassium chlorid was involved. In these experiments, no measurements of H ion or anion concentration were made so that a satisfactory analysis of the causes of the discrepancy is not possible. In the light of the conclusions quoted above, however, it seems probable that this inconsistency in our own results is more apparent than real and that the interchanges between added salts or solutions and soils in general are strictly stoichiometric, if all of the ions involved, including H or OH ion, are taken into account. To state that such interchanges take place in equivalent proportions and depend upon familiar chemical principles is satisfactory so far as it goes, but, because of the complexity of the systems involved, leaves a great deal to be learned as to the specific effects of salt or fertilizer treatments upon individual soils. The data referred to herein, as well as much other evidence that might be adduced, indicate a definite tendency toward an increase of water soluble constituents when salts are added to soils. This increase is obviously due, in part, to the added constituents which are not rendered insoluble by reaction with liquid or solid phase soil constituents and in part to new soluble material originating in the solid phase of the soil itself. Little is known as to the degree of permanence of the



new equilibria engendered by salt or fertilizer applications, and there is good reason to believe that these may be more or less transitory, particularly when the additions are small in magnitude. If this be true, the bearing of the factor of time upon ionic interchanges between soil and solution must be more completely studied if conclusions are to be drawn as to the ultimate effects of salt or fertilizer treatments on plant production. Moreover, the liquid phase of such treated soils must be studied concurrently with the growth of crops. The object of the present study was, therefore, to ascertain: first, the general qualitative and quantitative effects of salt and fertilizer treatments on the water extractable constituents of soils after the lapse of a considerable period of time (in this case eight months); and second, the effect of changes induced by salt and fertilizer treatments upon plant withdrawals and production. It is evident that comprehensive conclusions from this type of experimentation can be obtained only from a very large number of systematic studies of the effects of single salts on each of a great many soils. Such a method of attack is obviously beyond the facilities of any ordinary laboratory within a reasonable period of time, and we have deemed it necessary in the present state of our knowledge to use a small number of more or less complex treatments on a few soils. While such a procedure necessarily limits the conclusions, it may be expected to yield more facts and at the same time supply data for the proper formulation of systematic experiments of more restricted scope.

#### PRELIMINARY EXPERIMENTS

These consisted of a study of the effects of certain treatments on the water soluble constituents of two soils, one a silty clay loam known as No. 1c and one a sandy loam known as No. 15. The soil, after admixture, was kept for eight months in loosely covered jars permitting air circulation, the moisture content of the soils being maintained at 22 per cent and 15 per cent, respectively. The treatments included additions to each soil of (1) varied concentrations of a complete culture solution commonly used in this laboratory; (2) varied amounts of potassium nitrate and acid phosphate (superphosphate); and (3) certain amounts of tankage. The last named material, since it contained no water soluble constituents, was not expected to shed

light on chemical interrelations but was included because of the opportunity offered to compare its effects with those of the added salts.

No attempt was made to correlate the amounts of the various solutes added in the culture solution with those of field practice. It should be noted, however, that the amounts of constituents added in the minimum treatment with solutions (22 p.p.m. K, 57 p.p.m.  $\text{NO}_3$ , and 10 p.p.m.  $\text{PO}_4$ , corresponding approximately to 88, 308, and 40 pounds per acre foot), although fairly heavy for nitrate, would not constitute prohibitive applications in intensive fertilizer practice. Attention is called to this, not for the purpose of attempting to justify correlating the results of this experiment with field practice, but to indicate that the amounts of salts added in the minimum treatments were not so excessive as to constitute a highly artificial dosage. In this connection, we may also state that the minimum potassium nitrate-superphosphate and tankage treatments, while probably exceeding the economic limit under most circumstances, are not greatly in excess thereof.

The data obtained are presented in table 1.

#### EFFECT OF ADDED SALTS ON WATER EXTRACTABLE SOLUTES AFTER EIGHT MONTHS

In dealing with the results obtained by adding a mixture of salts to the soil complex, it is evident that one may not hope to completely differentiate between the effects of a given element or the single salt from which it is derived and those induced by reactions between the other added salts and the soil minerals. The effect of common ions in reaction has, however, such importance in determining solubilities as to require that the data be studied, in the first instance at least, in the light of the relation of specific elements in the added solutions to changes in the condition of the same elements in the soil.

We have attempted to classify the results obtained upon the basis of types of behavior in the relation between the amounts of solutes added and those extracted by water from the treated soils. Four distinct types of behavior may be noted:

1. The water extractable solute is increased in the treated soil, but the amount of the increase is not equal to the amount of the given solute in the added solution or salt.



Such behavior is shown by potassium and calcium in practically all instances in both soils; by nitrate in all instances in both soils with the exception of two treatments in soil 1c; and by magnesium and sulfate in soil 15. In all of these instances, except in the case of nitrate, the discrepancy between the amount of added solute and its increase in the water extract may be accounted for by removal of a

EIGHT MONTHS <sup>1</sup>											l.
											n
											y-
											e
											d
											d
											n
											a
So	m (Ca)		Magnesium (Mg)				Sodium (Na)				t
	Differ- ence	Added	Treat- ed soil	Un- treated soil	Differ- ence	Added	Treat- ed soil	Un- treated soil	Differ- ence	Added	d
1C	14	22	56	38	18	8	115	96	19	0	l-
	28	36	85	38	47	13	98	96	2	0	
	87	114	113	38	75	41	61	96	-35	0	y-
	21	39*	44	38	6	0	102	96	6	0	
	73	71*	60	38	22	0	107	96	11	0	
	132	226*	102	38	64	0	112	96	16	0	y
	28	.....	42	38	4	.....	107	96	11	.....	n
	41	.....	52	38	14	.....	110	96	14	.....	n
15	23	52	31	28	3	18	97	96	1	0	
	26	104	37	28	9	36	45	96	-51	0	
	130	329	69	28	41	116	72	96	-24	0	e
	40	263*	29	28	1	0	60	96	-36	0	-
	61	125*	29	28	1	0	80	96	-16	0	il
	182	225*	43	28	15	0	45	96	-51	0	il
	19	.....	24	28	-4	.....	146	96	50	.....	e
	28	.....	22	28	-6	.....	128	96	32	.....	e

in the solution added (41 p.p.m. Mg in soil 1c and 36 p.p.m. Mg. in soil 15—see also differences in sulfates in the two soils) as well as upon theoretical grounds, it is evident that the character of the solid phase material is largely determinative of whether the increase in amount of water extractable material is greater or less than that added in soluble form,

3. The water extractable solute is decreased by the treatment.

This applies to sodium in one instance in soil 1c and in numerous instances in soil 15. Such an effect upon a constituent capable of forming highly soluble salts demands further investigation, particularly in view of the inherent error in determining small quantities of this element. Literally interpreted, however, the results suggest and indeed can only mean that prolonged contact of soil and salt solutions may result in the formation of mineralogical species from which the sodium is less readily dissolved by water than from those present in the untreated soil.

4. The water extractable solute is increased by the treatments, but the increase constitutes only a small fraction of the added solute.

This appears to be the characteristic of phosphate behavior. With small applications, the increase is negligible or doubtful, but the larger applications induce appreciable increases in water soluble phosphate. More recent investigations<sup>3</sup> in this laboratory indicate that the soluble phosphate concentrations of natural soils fluctuate from time to time. This fluctuation may be ascribed to changes in reaction, or to changes in the concentrations of cations which tend to form insoluble phosphates. Obviously, the addition of soluble phosphates should tend to upset the phosphate equilibrium and it is not surprising that an increase of water extractable phosphate should ensue in spite of the low solubility of phosphates in general.

The addition of materials initially insoluble in water cannot be expected to furnish evidence as to the nature of ionic interchanges. Indeed, the results with tankage applications indicate that such materials have very little influence on the water solubility of soil constituents other than those contained in the added material itself. In the light of common experience in fertilizer practice, it is, however, not surprising that the nitrate and water soluble phosphate of soils should be increased by tankage applications.

#### VEGETATION EXPERIMENTS

The results of the experiments reported above demonstrated to our satisfaction that the potentially soluble constituents of soils may be readily increased for a considerable period of time and this by salt or fertilizer applications which are not necessarily unusual or excessive. They also gave valuable information as to the magnitude of the



increases in soluble matter to be expected from given applications, and thus served as a general guide to the treatment of these soils in studies of the relation of such increases to plant absorption and production. One of the soils used in the preceding experiment was included in the subsequent experiments where plants were grown on treated soils. This soil (No. 15) was at the time of the experiment in a high state of fertility and it was deemed desirable to include a soil of lower fertility. Soil 1c did not conform to this requirement and another soil known as No. 21 (Oakley blow sand) was substituted in its stead. On this account, the dosage used in the various treatments of this soil was not predicated on previous experimental work. It was known, however, that the soil does not yield good crops without substantial amounts of fertilizers.

*Procedure.*—Each soil was thoroughly screened and mixed, after which weighed portions received the various treatments in the form of solutions.

Each of the treated portions was again thoroughly mixed and placed in galvanized iron tanks 60 inches by 30 inches by 18 inches deep, according to the usual practice of this laboratory.<sup>6</sup> The crop, Beldi barley, was planted immediately after the soil was installed. The tanks were placed parallel to each other, with uniform exposure to light. All the soils were watered with distilled water and maintained at optimum moisture, each season, until the crop was nearly ripe. Only one treatment was made, but two successive crops were grown. The seasons covered were 1920 and 1921 for soil No. 15, and 1921 and 1922 for soil No. 21. Water extractions of both soils under all treatments were made periodically for nearly two years, but the periods were less frequent the second year.

*Soil Treatments.*—From the results of our preliminary experiment, it is evident that while all soluble salts are likely to have important effects on the solubility of soil constituents, increases of specific solutes can be most readily and certainly brought about and maintained if the particular solute which it is desired to increase is added to the soil. On this account, in vegetation experiments of limited scope, it is desirable to treat the soil with salts capable of supplying those ions which are not only readily absorbed by the plant, but which are required in substantial quantity for its development. Such ions obviously include nitrate, phosphate, and potassium, and these were used in the form of

sodium nitrate, sodium dihydrogen phosphate, and potassium chlorid. In the treatment of soil No. 15, the attempt was made to use such amounts of the various salts as would about double the amounts extractable by water from the untreated soil. Since the amount of fixation of phosphate and potassium varies with the time of contact of solution and soil, and since losses of nitrate through biological activities are to be expected, it is obvious that attempts to double the water extractable constituent could at best only result in an approximation thereto and this for a limited period. The results, hereafter, show that we have been reasonably successful in approximately doubling the water extractable nitrate and potassium in the early stages of the experiment, but that owing to an over-estimate of the fixing power of the soil for phosphate, the addition of phosphate was, perhaps, unnecessarily large. When, as a result of the work of the first season, an additional experiment with a soil of low fertility was decided upon, it appeared necessary from what was known of the soil selected (No. 21), to add very much larger quantities of nitrate in its treatment if an adequate response in crop production was to be expected. The amount of the potassium salt treatment of this soil was left as before and the phosphate treatment diminished as suggested by the results of the work of the preceding year with soil No. 15.

The schedules of treatment are indicated in the following tables:

TABLE 2A  
SCHEDULE OF TREATMENTS AND YIELDS OF SOIL No. 15

Tank No.	Designation	Treatments in terms of:		Air-dry weight of crops (Grams per tank, 12½ square feet)	
		Pounds per acre foot	P. P. M. of water-free soil	1920	1921*
1	Half nitrate.....	111 NaNO <sub>3</sub>	20 NO <sub>3</sub>	830	Discontinued
2	Nitrate.....	221 NaNO <sub>3</sub>	40 NO <sub>3</sub>	895	475
3	Nitrate-Phosphate.....	221 NaNO <sub>3</sub>	40 NO <sub>3</sub>		
		1010 NaH <sub>2</sub> PO <sub>4</sub>	198 PO <sub>4</sub>	1071	552
4	Untreated.....	.....	.....	811	425
5	Phosphate.....	1010 NaH <sub>2</sub> PO <sub>4</sub>	198 PO <sub>4</sub>	908	436
6	Half phosphate.....	505 NaH <sub>2</sub> PO <sub>4</sub>	99 PO <sub>4</sub>	921	Discontinued
7	Potassium.....	768 KCl	100 K	816	Discontinued
8	Half potassium.....	384 KCl	50 K	844	Discontinued

\* The crop was attacked by a fungus disease and the low yields in this column are not regarded as conclusive of a falling off in fertility (see results from Soil No. 21 for such an effect).



TABLE 2B  
SCHEDULE OF TREATMENTS AND YIELDS OF SOIL No. 21

Tank No.	Designation	Treatments in terms of:		Air-dry weight of crops (Grams per tank, 12½ square feet)	
		Pounds per acre foot	P. P. M. of water-free soil	1921	1922
1	Nitrate.....	554 NaNO <sub>3</sub>	100 NO <sub>3</sub>	1144	418
2	Nitrate-phosphate.....	554 NaNO <sub>3</sub> 630 NaH <sub>2</sub> PO <sub>4</sub>	100 NO <sub>3</sub> 124 PO <sub>4</sub>	1272	407
3	Untreated.....	.....	.....		
4	Phosphate.....	630 NaH <sub>2</sub> PO <sub>4</sub> ....	124 PO <sub>4</sub>	799	134
5	Potassium.....	768 KCL	100 K	820	306
6	Untreated, uncropped	.....	.....	.....	.....

DISCUSSION OF RESULTS

The discussion of results is conveniently divided into two parts: first, a consideration of the effects of the added salts on the condition of the various elements in the soil from time to time as indicated by the results of water extraction; second, such correlations as may appear between the amounts of the various solutes actually absorbed by the crop and the amounts found by water extraction of the soil on the one hand, or by the amounts of dry matter production on the other. This division permits of the separate graphic representation (figs. 1 to 5) of the effects of salt additions on water extractable soil constituents and of a brief tabular presentation of the results of absorption by the crop (tables 3A and 3B).

In considering the graphic representation of effects on the soil, it should be understood that the results from the minimum applications (half nitrate, half phosphate and half potassium) are omitted from the graphs in order to avoid confusion in following the lines representing different treatments. Since these data are not included elsewhere, it is necessary to explain that these smaller treatments generally produced qualitative effects similar to those brought about by the larger applications, but that these effects were uniformly of smaller magnitude.

In further explanation of the graphs, attention should be drawn to the fact that the work with soil No. 15 did not include an untreated uncropped, or fallow check. To those who are unfamiliar with the



previous work from this laboratory<sup>6</sup> covering the behavior of water extractable solutes in cropped and uncropped soils without fertilizers, it should be stated that uncropped soils invariably maintain themselves at higher levels than do their cropped duplicates.

In the work with soil No. 21, we have included an untreated, uncropped plot which shows the typical behavior of fallowed soils. Attention should also be called to the fact that in the season of 1922, the second year of the experiments with soil No. 21, the number of observations made were extremely limited, so that changes in direction of the lines of the graphs are doubtless more abrupt than a larger number of determinations would have shown.

*Nitrate in soils.*—An important characteristic of nitrate behavior in soils and one which has received much attention in this laboratory is that this constituent is invariably reduced to a low level and practically disappears from cropped soils at the height of the growing season (plants being 8–9 weeks old). Such an effect is to be observed in the present data in both soils for both seasons in the lines representing nitrate in the cropped, but otherwise untreated soils. While the evidence is overwhelming that nitrates tend to be reduced to a very low level in soils under crop, it is by no means certain that soils carrying very large amounts of nitrates at the beginning of the growing season or so constituted biologically as to resist losses of nitrate nitrogen, may not be able to retain substantial concentrations of nitrates throughout the growing season. The present data show, however, that considerable increases of the initial nitrate concentrations do not prevent the practical disappearance of nitrate in soils under crop. Such enhanced concentrations persist for a comparatively short time, and diminish to negligible amounts at the height of the growing season. After the harvesting of the crop, the nitrate content of the soil again increases and, independently of the previous treatment, reaches a maximum in the spring of the following year. Then, in the absence of further treatments, the nitrate behavior appears to be identical in all plots of both soils. None of the treatments appear to have substantially changed the nitrate concentrations at any time with the exception of those carrying additional nitrate and these only affect the soil for a very limited period.

*Phosphate in soils.*—The fact that the soil solution of most natural soils is probably saturated with reference to phosphate in the particular system and that the phosphate concentration of such solutions is independent of the amount of moisture present, now seems quite certain.<sup>2</sup> This does not mean that the concentrations of phosphate in a given soil may not vary from time to time. Indeed, the converse is to be expected if the reaction of the soil is modified by any cause or if there occur substantial changes in the concentration of certain cations in the liquid phase. Changes in concentration of cations capable of forming comparatively insoluble phosphates (Ca, Mg) may be brought about in cropped soils by plant absorption, by leaching, and by addition of salts tending to change the previous equilibrium. It is evident, however, that fluctuations in phosphate concentration, if small in magnitude, may not be measured with certainty by the criterion (water extraction) used in the present work. It is not surprising, therefore, that significant variations of water extractable phosphate do not appear in either of our soils, whatever the treatment, in the absence of applications of large amounts of soluble phosphate. Where such additions were made, however, the water extractable phosphate has been greatly increased over a period of two years, although it is gradually but certainly diminishing. We interpret this to mean that the equilibrium of the system, soil-soil moisture, has been modified by these large applications of sodium-dihydrogen-phosphate, but that unless the nature of the solid phase material, and particularly of the undissolved phosphates therein, has been substantially modified by the treatment, the phosphate concentrations in the soil solution will ultimately return to their former magnitudes, whatever these may have been.

*Potassium in soils.*—Addition of potassium chlorid equivalent to 100 p.p.m. of soil approximately doubled the initial water extractable potassium in both soils, which, with fluctuations, decreased somewhat at the end of the first year. The results of other treatments do not appear to have materially affected the water extractable potassium in soil No. 15. In soil No. 21 the treatments carrying phosphate diminished the water extractable potassium from the first, and this effect persisted for the entire subsequent period (nearly two years) of observation. Water extractable potassium was also decreased in the nitrate treated soil after the first few weeks, when the plants had

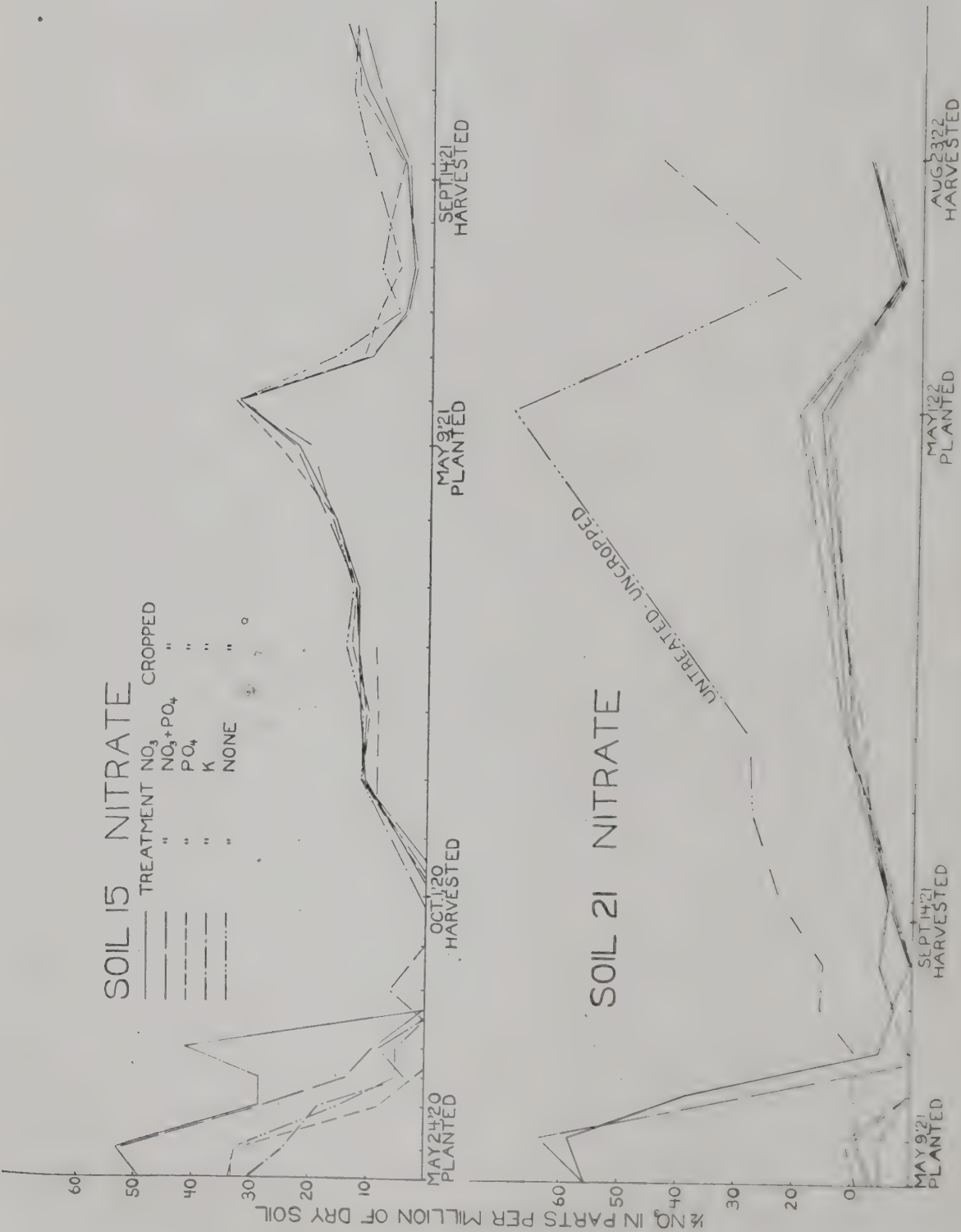


Fig. 1



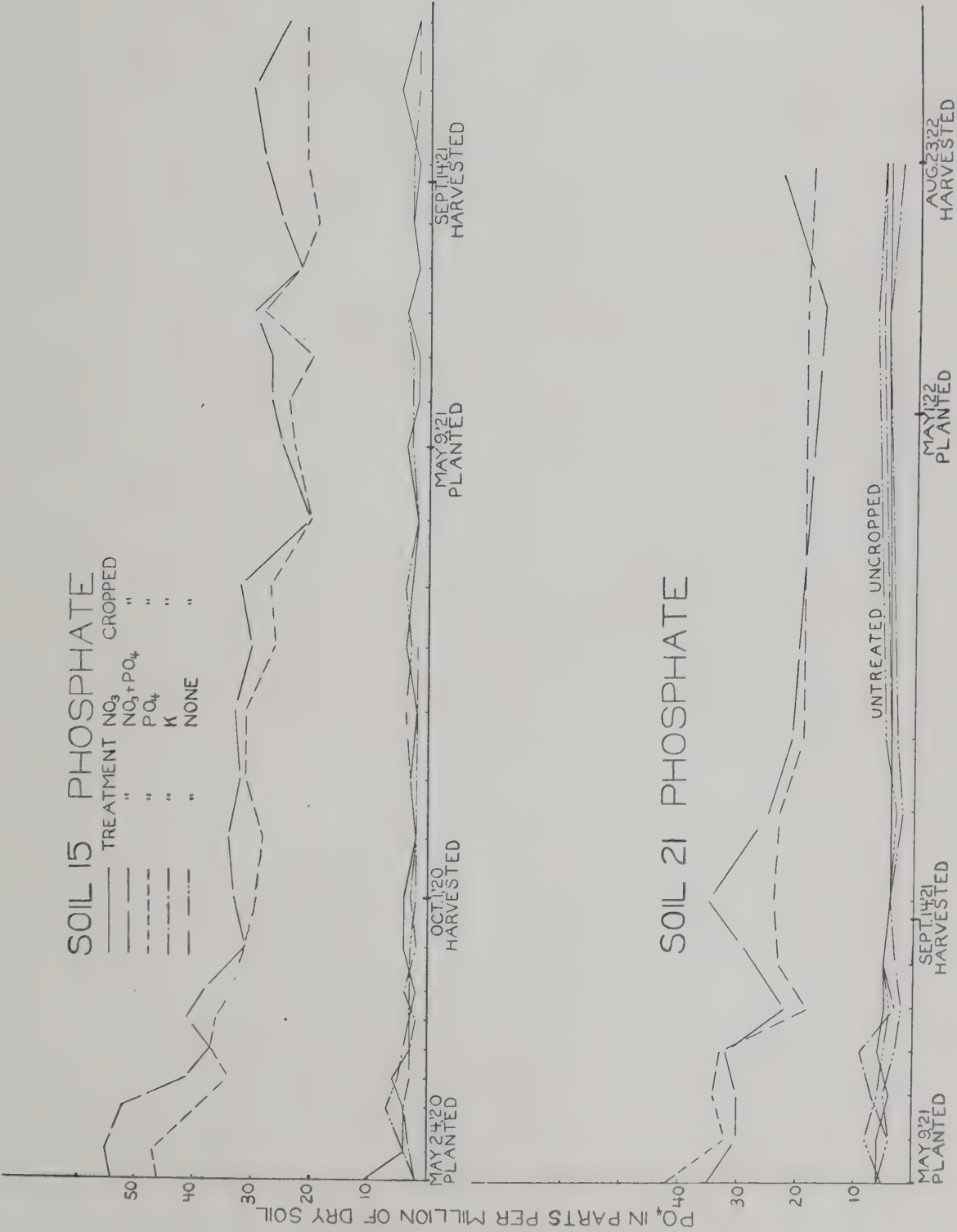


Fig. 2

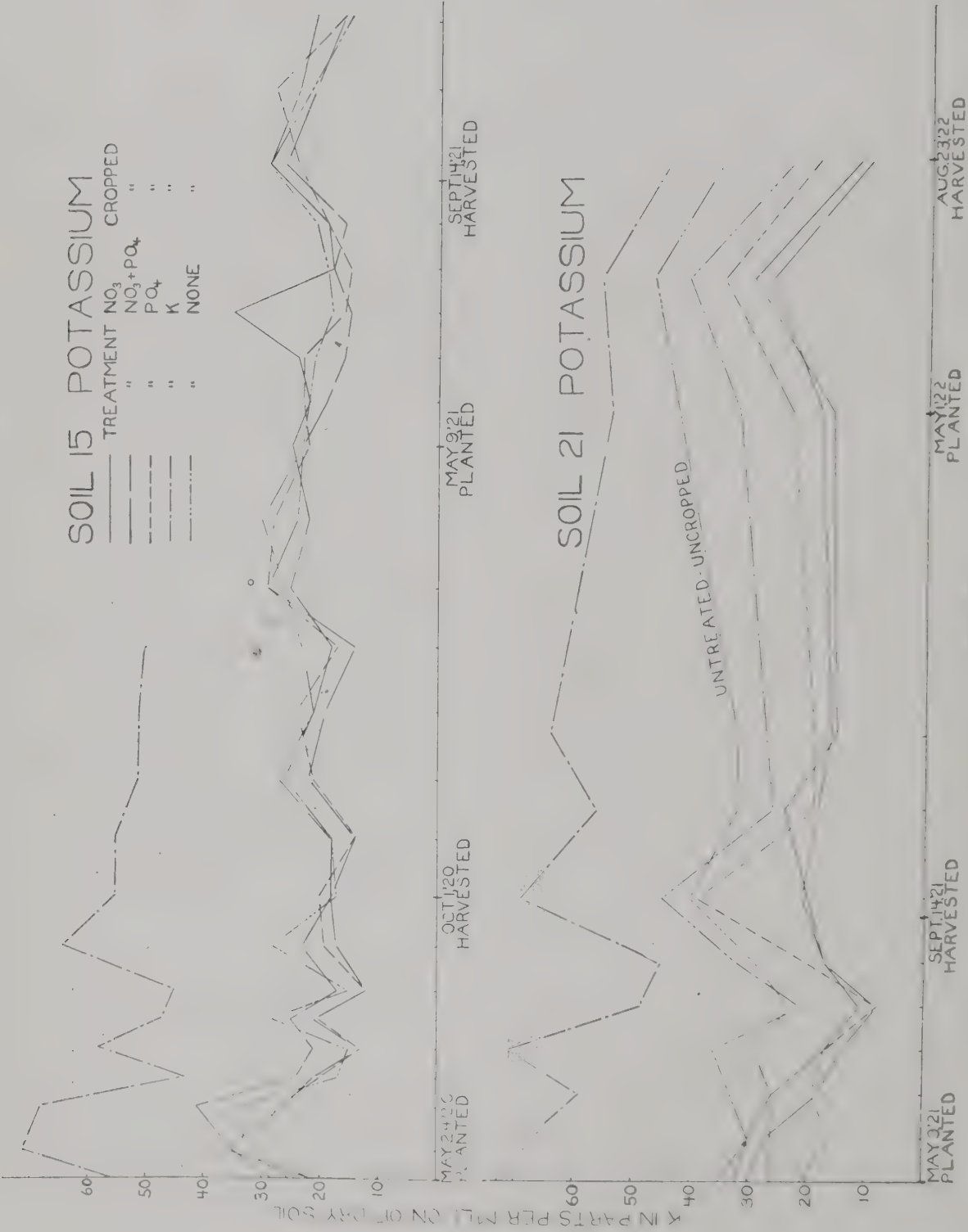


Fig. 3

reached a considerable degree of development and were doubtless actively drawing upon the potassium of the soil.<sup>1</sup> With soil No. 21 the actual amounts of potassium withdrawn from the nitrate treated and the nitrate-phosphate treated portions (table 3B) were considerably greater than those from the untreated portion and suggest that the diminished amounts of water extractable potassium found in these soils after the crop was well grown might be directly related to the growth of the crop. This would not, however, account for the diminished amount of water extractable potassium found in both of the phosphate treated portions of soil No. 21 in the first few weeks after treatment, nor to the persistently low figures obtained subsequently from the portion of soil where phosphate alone was used in the treatment and where the withdrawal of potassium by the plants was not very much larger than that from the untreated portion of soil. We can only suggest that in spite of the comparatively high solubility of potassium phosphate, there may be a partial precipitation of potassium when phosphate is added to certain equilibrium systems. This might reasonably be expected to be reflected in diminished amounts of water extractable potassium.

*Calcium and magnesium in soils.*—Since neither calcium nor magnesium salts were added to the soils, it is evident that observed changes in water extractable calcium and magnesium were caused either by plant withdrawals of these elements or by reactions between added salts and soil constituents, or by changed solubility of solid phase material in the new soil solution. Where potassium salts were added to the soils, there was a substantial increase in water extractable calcium in both soils and of water extractable magnesium in soil No. 15. This increased solubility began before the effect of plant withdrawals had had an opportunity to operate and is definitely referable to chemical interactions with the soil. The other treatments, in general, gave lower results for water extractable calcium and magnesium than did the untreated cropped soil, which taken alone, might suggest that great absorption by the plants had depressed the amounts of calcium and magnesium capable of being extracted by water. It should be noted, however, that the depression of these elements so determined was generally and substantially lower in the case of those treatments which included phosphate, than in that of the nitrate treatment, although in one soil (No. 21), the nitrate treated portion



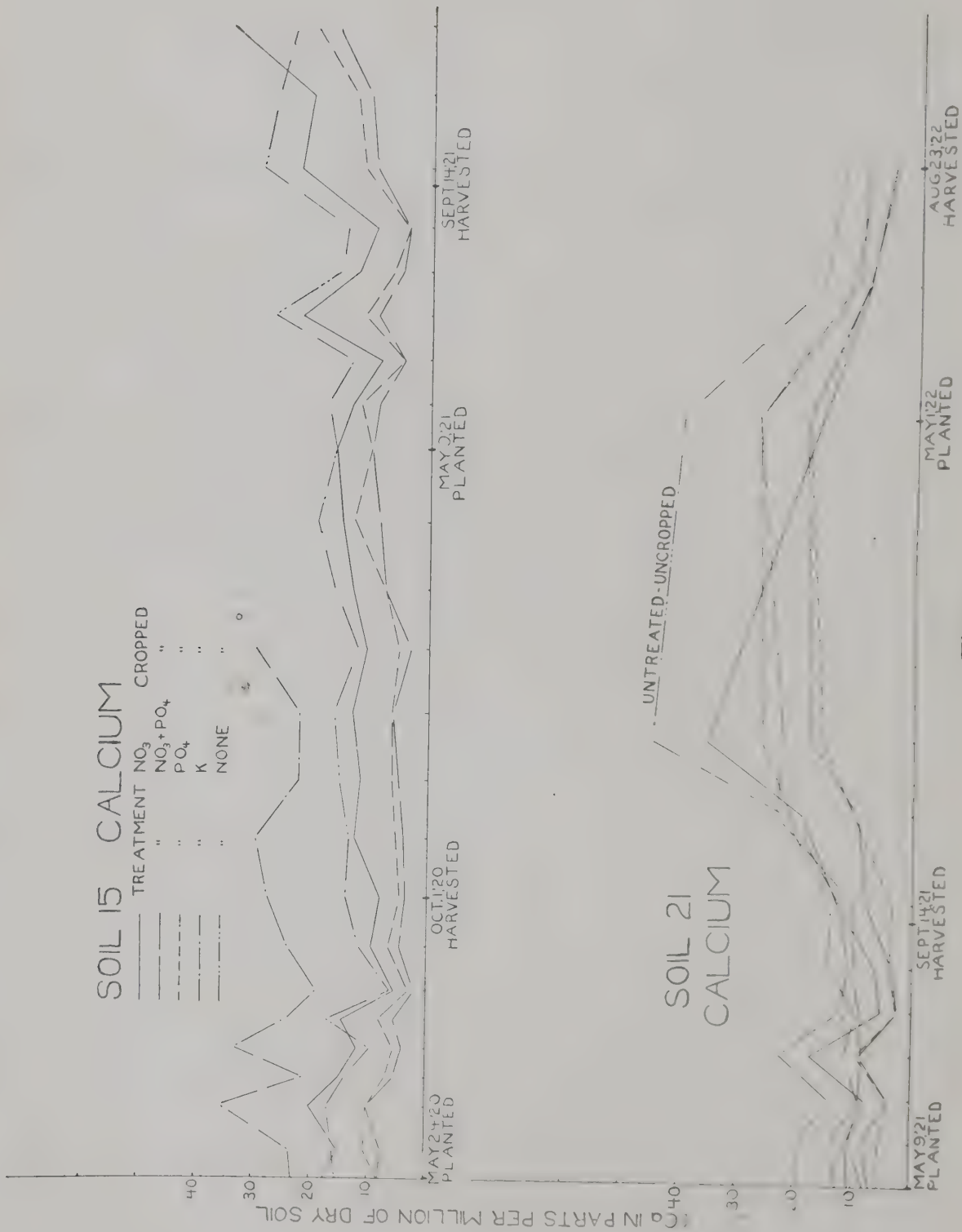


Fig. 4

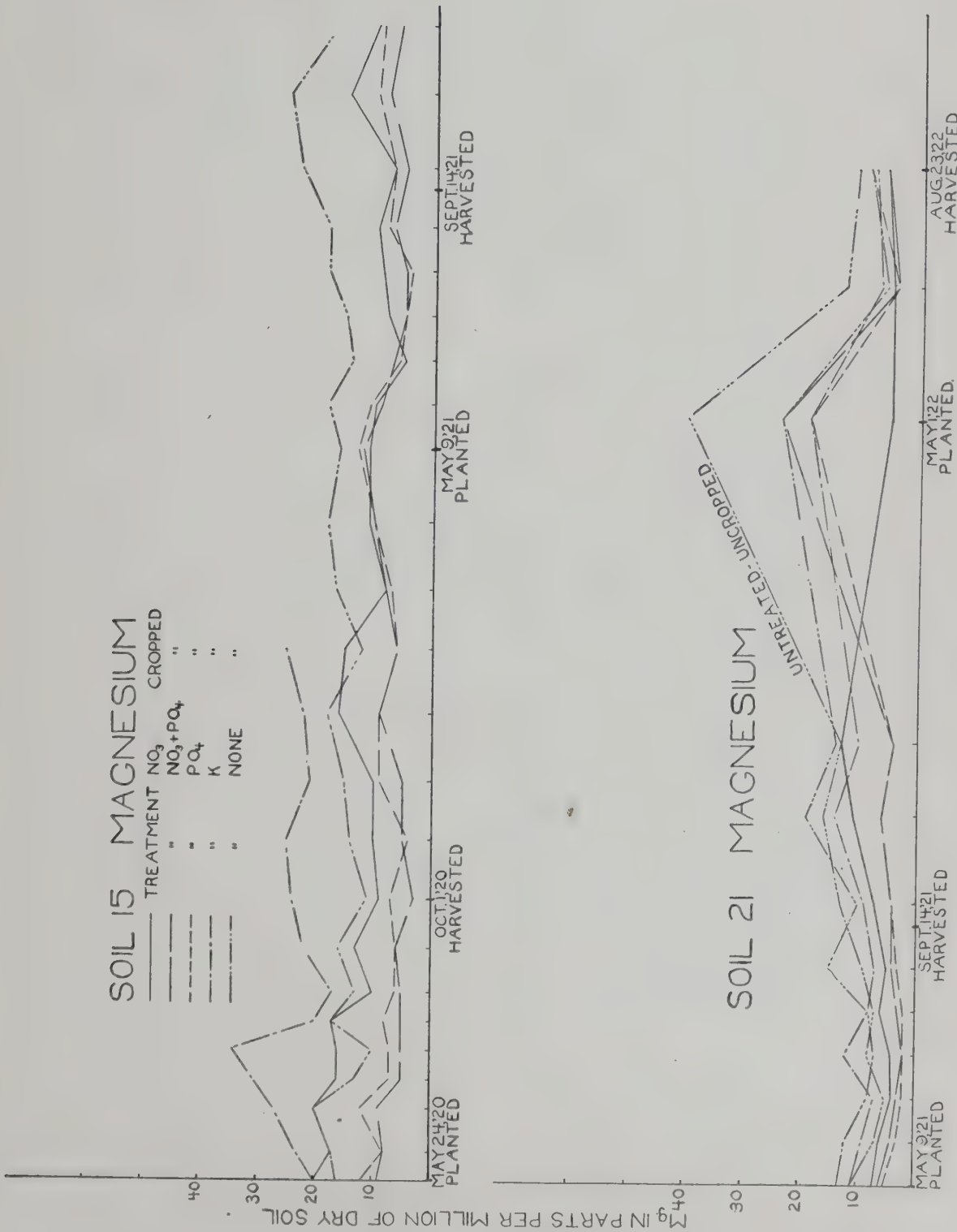


Fig. 5



yielded crops which absorbed greater absolute amounts of calcium and magnesium than did those receiving phosphate alone (see table 3B). This inconsistency and the small absolute values for calcium and magnesium withdrawn by the crops from both soils render it probable that interactions of calcium and magnesium with added phosphate have been the principal cause of the depression noted above. It is difficult to avoid the conclusion that a similar correlation must have existed in the true soil solution and that there is a very definite reciprocal relation between phosphate concentration and the amounts of calcium and magnesium in the liquid phase.

#### RELATION OF PLANT WITHDRAWALS TO CHANGES IN WATER EXTRACTABLE CONSTITUENTS

That a given element may be absorbed in greater quantity by a large and strong plant than by a less developed one upon a common substratum of soil or solution is generally recognized. It is also well known that if greater growth is obtained by the removal of some limitation, such° as nitrogen deficiency, the plant usually absorbs larger quantities of other elements which do not themselves constitute limiting factors. It is evident that the causes of increased absorption cannot be dissociated where changes are brought about in the soil by addition of solutes or by other means, unless the crops obtained are relatively uniform in total yield of plant material. This result, obviously, cannot be brought about unless the limiting factor is one such as sunlight, which is extraneous to the soil itself. These considerations appear to require that we use a fertile soil in attempts to correlate plant withdrawals with changes in solubility of soil constituents.

Soil No. 15, considered above in relation to the changes brought about by additions of solutes, answers this requirement and when cropped actually did give relatively constant yields in seven, out of eight, different treatments. The one exception in which there was what appears to be a substantial increase in yield, due to changes in the solubility of soil constituents, was that in which both nitrate and phosphate were added to the soil, but no single so-called fertilizer constituent had that effect.

TABLE 3A  
WITHDRAWALS OF IMPORTANT CONSTITUENTS FROM VARIOUSLY TREATED SOIL BY 105 BARLEY PLANTS  
(Figures represent grams per tank)\*  
Fertile Soil No. 15. Season of 1920

Tank No.	Treatment Grams per tank	**Effect of treatment on water extractable constituents of the soil	Yield water-free dry matter	Constituents withdrawn				
				Nitrogen (N)	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)
1	Nitrate of soda, 16 NO <sub>3</sub> .....	+NO <sub>3</sub> , = PO <sub>4</sub> , = Ca, = Mg, = K.....	800	12.65	3.67	11.55	2.81	1.55
2	Nitrate of soda, 33 NO <sub>3</sub> .....	+NO <sub>3</sub> , = PO <sub>4</sub> , = Ca, = Mg, = K.....	862	14.44	3.86	12.52	3.52	1.76
4	Untreated.....	None.....	786	11.16	3.34	10.21	2.42	1.33
5	Sodium-Hydrogen-Phosphate 162 PO <sub>4</sub> .....	= NO <sub>3</sub> , + PO <sub>4</sub> , - Ca, - Mg, = K.....	877	9.81	5.20	9.05	3.03	1.19
6	Sodium-Hydrogen-Phosphate 81 PO <sub>4</sub> .....	= NO <sub>3</sub> , + PO <sub>4</sub> , - Ca, - Mg, = K.....	894	10.43	4.00	9.59	2.09	1.05
7	Potassium chloride, 82 K.....	= NO <sub>3</sub> , = PO <sub>4</sub> , + Ca, + Mg, + K.....	792	10.80	3.34	12.56	2.78	1.30
8	Potassium chloride, 41 K.....	= NO <sub>3</sub> , = PO <sub>4</sub> , + Ca, + Mg, + K.....	818	10.26	3.21	12.74	2.10	1.28
3	Nitrate of soda, 33 NO <sub>3</sub> Sodium-Hydrogen-Phosphate 162 PO <sub>4</sub> .....	+NO <sub>3</sub> , + PO <sub>4</sub> , - Ca, - Mg, = K.....	1043	15.54	5.52	9.24	2.97	1.64

\*\* + means increase of water extractable constituents due to the treatment.  
- means decrease of water extractable constituents due to the treatment.  
= means no substantial change of water extractable constituents due to the treatment.  
These estimates are based upon behavior during the early part of the growing season and before the withdrawals by the crop had an opportunity to affect the results.  
\*Figures for treatments and withdrawals may be converted into terms of P. P. M. of soil by multiplying by 1.23  $\left(\frac{1,000,000}{1800 \times 453}\right)$ , since each tank contained 1800 pounds of soil.



If we exclude from present consideration the data obtained from the soil in tank 3, nitrate-phosphate application (table 3A), the average yield of water-free dry matter is 833, the maximum is 894, and the minimum 786, grams per tank. No statistical study of the weights of individual plants was made, but these small variations in total yield probably fall within the limits of variability in the plants.

When we come to examine the absolute amounts of individual constituents withdrawn from each tank, it is by no means clear that many of the small differences observed represent significant variations, due to treatment. Another difficulty presents itself in that, while the water extractable constituents probably represent the soil solution, they are not a very precise measure of its concentration for constituents other than nitrate. For these reasons, only the most definite and apparently consistent relations between the effects of treatment and plant withdrawals will be noted.

#### COMPARISON OF WITHDRAWALS WITH EFFECTS OF TREATMENT WHEN NO INCREASE IN YIELD WAS OBTAINED

(Table 3A, excluding data from tank No. 3)

*Nitrate.*—In both cases where water extractable nitrate was increased in the soil, the withdrawal of nitrogen by the plant was increased more than with any other treatment, but without substantial increase in yield. Even if the differences in yield are regarded as significant, the nitrogen withdrawal is more than proportional thereto and the increased absorption of this element may thus be ascribed with a considerable degree of assurance to the increase of nitrate in the soil and not to a larger growth of the plants.

*Phosphorus.*—In both cases, where the water extractable phosphate of the soil was increased, the withdrawal of phosphorus was increased. If the differences in yield of crop are regarded as significant, the withdrawal of phosphorus is more than proportional thereto and apparently reflects the effect of the treatment on the soil. In two other cases (tanks 1 and 2), however, the phosphorus withdrawal is also somewhat greater than that from the untreated soil and slightly more than proportional to the actual differences in yield. No increase in water extractable phosphate has been brought about by these treatments and the correlation fails. It would appear either that the



difference observed is not significant or that phosphorus withdrawal from the portions of soil treated with sodium nitrate has been facilitated by changes in the soil solution which are not made evident by the present data.

*Potassium*.—The potassium withdrawal by plants in both cases of soil treated with potassium chlorid was greater than that from untreated soil, and may be reasonably ascribed to the increased amounts of water extractable potassium observed in the soil. It is also to be noted, however, that the same effect was produced by sodium nitrate treatment without a corresponding increase in water extractable potassium of the soil. The well known power of sodium to replace potassium in the solid phase suggests that there may have been a slight increase of potassium concentration in the soil solution which is not definitely reflected in the water extracts. On the other hand, the phosphate treatments involving much larger additions of sodium to the soil than was the case in the nitrate treatments, not only did not increase but actually decreased the potassium withdrawals. One explanation of this apparent anomaly is suggested by the fact that in the phosphate treatments the water extractable calcium and magnesium were lowered, and it is not impossible that potassium in the true soil solution has been decreased, although the effect is not definitely demonstrated by water extraction of the soil upon which these crops were grown. Another possible cause is suggested by recent work<sup>4</sup> in which it has been shown that increased sodium concentrations diminish the absorption of potassium from culture solutions.

*Calcium and magnesium*.—No uniformly consistent relation was shown between increases or decreases in water extractable calcium and magnesium, and withdrawals by the plant. Increased withdrawals of calcium were found when the water extractable calcium of the soil remained substantially unchanged and when it was slightly increased or diminished. Decreased withdrawals were also found when the water extractable calcium was slightly increased, and when it was somewhat diminished by the treatments. Magnesium shows increased withdrawals when the water extractable magnesium remained substantially unchanged, and diminished withdrawals when the water extractable magnesium was slightly diminished. In two cases, a slight increase in water extractable magnesium was noted without apparent effect upon withdrawal.

COMPARISON OF WITHDRAWALS WHEN AN INCREASED YIELD  
WAS OBTAINED

When an increase in yield was obtained (table 3A, cf. tanks 3 and 4), there was an increase in withdrawal of all constituents, except potassium. The increased withdrawal of nitrogen and phosphorus is clearly referable to the large increases in water extractable nitrate and phosphate. The water extractable calcium and magnesium have been diminished by the treatment, but the increased withdrawals of these constituents can be accounted for by the increased size and absorbing power of the crop. The diminished absorption of potassium by the larger crop must be accounted for by such reasons as have been suggested heretofore in the consideration of data from the crops where no increases in yield were obtained.

When we turn to the results from an infertile soil, it is at once evident that the withdrawal of soil constituents as a whole was more closely correlated with the yields than with changes in amount of water extractable constituents. Thus increased yields have enabled the plant to take up significantly larger quantities of calcium and magnesium when these were neither added to the soil nor increased in concentration by indirect methods as indicated by water extraction. Moreover, instances occur where substantial increases in withdrawal of the elements occur with diminution of water extractable calcium and magnesium. In the case of potassium, there are two instances where larger quantities of this element are removed from the soil when the water extractable potassium remained relatively constant than when large amounts of potassium chlorid were added to the soil and a very great increase in water extractable potassium was developed.

In all cases where increased yields were obtained, phosphorus withdrawal was substantially greater than that from the untreated portion of soil. When a large yield was obtained from soil in which no increase of water extractable phosphate was induced by the treatment (tank 1), there was as much phosphorus withdrawn from the soil as where a substantial increase of water extractable phosphate was brought about by treatment (tank 4), but where the yield of crop was not so great. The only clear correlation between withdrawals and the amounts of water extractable constituents in this soil is in the case of nitrogen. It is evident that the soil under discussion is one which



TABLE 3B  
WITHDRAWALS OF IMPORTANT CONSTITUENTS FROM VARIOUSLY TREATED SOIL BY 105 BARLEY PLANTS  
(Figures represent grams per tank)\*  
Infertile Soil No. 21. Season of 1921

Tank No.	Treatment Grams per tank	**Effect of treatment on water extractable constituents of the soil	Yield water-free dry matter	Constituents withdrawn				
				Nitrogen (N)	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)
3	Untreated.....	None.....	306	4.63	1.21	4.59	1.31	0.48
5	Potassium chloride, 81 K.....	+NO <sub>3</sub> , = PO <sub>4</sub> , +Ca, = Mg, +K.....	628	6.10	2.18	9.68	2.28	1.12
4	Sodium-Hydrogen-Phosphate 101 PO <sub>4</sub> .....	+NO <sub>3</sub> , +PO <sub>4</sub> , -Ca, -Mg, -K.....	630	6.32	3.76	6.62	2.10	0.95
1	Nitrate of soda, 82 NO <sub>3</sub> .....	+NO <sub>3</sub> , = PO <sub>4</sub> , = Ca, -Mg, = K.....	1035	16.45	3.82	13.27	3.69	1.34
2	Nitrate of soda, 82 NO <sub>3</sub> , Sodium-Hydrogen-Phosphate, 101 PO <sub>4</sub> .....	+NO <sub>3</sub> , +PO <sub>4</sub> , -Ca, -Mg, -K.....	1144	18.40	6.25	12.58	3.85	1.54

\*\*+means increase of water extractable constituents due to the treatment.

—means decrease of water extractable constituents due to the treatment.

=means no substantial change of water extractable constituents due to the treatment.

These estimates are based upon behavior during the early part of the growing season and before the withdrawals by the crop had an opportunity to affect the results.

\*Figures for treatments and withdrawals may be converted into terms of P P. M. of soil by multiplying by 1.23  $\left(\frac{1,000,000}{1800 \times 453}\right)$ , since each tank contained 1800 pounds of soil.



is deficient in power to supply nitrogen to the plant, but that almost any salt treatment stimulates nitrification, a result which is reflected in the yield of the crop, and in the power of the crop to acquire other elements even though these latter are diminished in solubility in the soil. This lack of correlation between withdrawals of elements which are not primarily limiting growth and the solubility of these same elements in the soil can hardly be charged to the inadequacy of water extraction as a criterion of that solubility. Probably no one will insist that diminished figures for water extractable constituents connote an increase in concentration of such constituents in the soil solution; yet such diminished figures frequently appear concurrently with increased withdrawals. The cause of increased withdrawals in such cases is to be sought in the greater total absorbing power of the plants due to a larger growth. We do not suggest that withdrawals of constituents which appear to be quantitatively of secondary importance in growth, are not a factor in growth; but it would appear that the amount of growth, however produced, is a preponderating element in causing an increased withdrawal of such constituents from the soil.

#### EFFECTS OF TREATMENTS AND WITHDRAWALS UPON SUBSEQUENT CROPS

The data covering yields of crops the second season after treatment have been presented (see tables 2A and 2B). Unfortunately, as stated in the footnote to table 2A, the second year's crop on soil No. 15 was attacked by a fungous disease which vitiates the data for that year. The effect of the first year's treatment and withdrawals on the second year's yield is shown by the data from soil No. 21 (table 2B). Here, it will be observed, there was a falling off in yield from all plots of soil whatever the treatment. The yield of the untreated soil has fallen off consistently with its known infertile character. One of the plots (tank 4), which gave a fairly large yield the first year under the influence of treatment, apparently suffered the second year because of the increased withdrawals by the preceding crop. The plots which received nitrate and produced large crops the first year retained their relative superiority the second year, but the amount of crop then produced was probably not significantly greater than that of the untreated plot the first year. When an infertile soil produces such a small absolute yield, it is dangerous to draw inferences from what appear to

be considerable differences in relative yield. The most that can be said under the circumstances is that the greater withdrawals from tanks 1 and 2 the first year have not prevented a relatively good yield the second year (as compared with the untreated plot). It will be recalled, however (fig. 1), that differences in nitrate content of all of the soil disappear the first year and it is evident that the superiority of the nitrate treated plots the second year, if regarded as significant, is not due to any residuum of nitrate remaining in the soil, but to indirect effects of the reaction of the salt sodium nitrate on the soil.

### SUMMARY

1. Two soils treated with various solutions and salt mixtures, when examined after eight months, showed substantial increases in water extractable constituents in general. In most cases, the increase of a given constituent was less than the amount of solute added, but magnesium and sulfate in one of the two soils increased more than could be accounted for by the addition of these elements.

2. The changes induced are ascribed to added solutes, to chemical replacements of solid phase material, to fixation by the solid phase, and to increased solubility of solid phase material in the new soil solution.

3. Two soils upon which crops were grown received various treatments of nitrate of soda, sodium dihydrogen phosphate, and potassium chlorid. Increase of the added solute was always observed in water extracts of the soil in the early part of the season. Additions of nitrate, however, did not prevent the practical disappearance of this ion at the height of the growing season. A reciprocal relation between added phosphate and other solutes is made evident, there being diminished amounts of water extractable calcium and magnesium in both soils, and of potassium in one soil when the water extractable phosphate was increased by treatment.

4. When no increase of crop was brought about by treatment of the soil, a correlation was observed between increases in given constituents in the soil as measured by water extraction (1-5), and the withdrawals of such constituents by the plants. On the other hand, increased or diminished withdrawals were frequently observed which bore no apparent relation to increased or diminished amounts of water

extractable constituents. This result may be ascribed to the defects of water extraction as a measure of the soil solution or to the influence of changed concentrations of other constituents upon the absorption of a given element by the plant.

5. When an increase of crop was brought about by treatment of the soil, there was a definite correlation between withdrawals of some constituent, or constituents (nitrate and phosphate in soil 15, nitrate in soil 21), and the yields of dry matter obtained; but the amounts of other constituents withdrawn appear to have been determined either by changed concentrations, or by the increased amounts of dry matter produced.



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REPLACEABLE BASES IN SOILS

BY

WALTER P. KELLEY AND S. MELVIN BROWN

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### INTRODUCTION

Former papers from this laboratory<sup>7, 22, 23</sup> have set forth the results of studies on the effects of salts on soils. In harmony with many previous workers we have shown that the reactions which take place when neutral salt solutions are used are of the nature of an exchange of bases; a part of the base of the solution is absorbed by the soil and some one or more of the bases of the soil pass into the solution. The exchange of bases is stoichiometric, or approximately so. The bases thus brought into solution are referred to as being replaced. With neutral or alkaline soils these bases are mainly calcium, magnesium, potassium and sodium, but as will be shown later acid soils may contain still other replaceable cations.

Two hypotheses, one chemical and the other physical, have been advanced to account for these reactions. The first was proposed by Way<sup>41</sup> in 1852 as a result of his studies on certain artificially prepared aluminosilicates. Twenty-five years later van Bemmelen<sup>37</sup> accepted the essentials of Way's views and adopted the suggestion previously made by others that the compounds involved in the replacement reactions are of a zeolitic nature. As is well known van Bemmelen<sup>38</sup> abandoned this view about ten years later as a result of his studies on colloidal materials. He then concluded that the replaceable bases are not chemically combined with the soil but are present in a state of adsorption. This latter view has since been quite widely accepted, although not universally so.

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\* Paper No. 119, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.



The fact that soil has the power of absorbing potassium and ammonium, a power which enables it to retain these constituents of fertilizers against the leaching action of rains, has attracted the attention of soil workers for three-quarters of a century.\* Largely because of the dominance of the so-called plant-food doctrine, comparatively little study has been devoted until recently to the relationships between the replaceable bases and the general chemical and physical properties of the soil mass. Soon after taking up a study of this subject about six years ago the senior author became convinced that the effects produced by soluble salts as a result of replacement reactions may have far-reaching consequences. Recent publications from this and other laboratories<sup>7, 8, 11, 20, 23, 31</sup> have abundantly confirmed this view. We may assert with confidence that significant changes take place in certain of the solid components of soils as a result of the substitution of one base for another.

It now seems certain that some of the most difficult phases of the alkali problem of semi-arid regions are closely related to and indeed caused by the substitution of sodium for one or more of the bases normally present in replaceable form. There are two especially important effects produced by the substitution of sodium for the divalent bases, namely: (1) the granular structure of the clay materials becomes broken down with the resulting development of extreme impermeability; (2) sodium carbonate is formed as a result of hydrolysis. The second of these effects has been discussed by Cummins and Kelley<sup>7</sup> and others;<sup>8, 11, 31</sup> the first will be the subject of a later communication (see 12).

A moment's reflection suggests that those alkali soils which contain the greatest amount of replaceable bases are likely to contain the greatest amount of absorbed sodium. Our studies have shown this to be true. We now know that in the reclamation of alkali soils the absorbed sodium may necessitate the application of special treatments. While this fact has been emphasized by several workers in this field, it is doubtful whether many students of alkali soils fully appreciate its significance.

The replaceable bases of soils which do not contain high concentrations of soluble salts also perform important functions which have

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\* It seems unnecessary to give an extended bibliography of this subject. Those who are interested in its historical development should consult papers by Patten and Waggaman,<sup>29</sup> Sullivan,<sup>34</sup> Prescott<sup>30</sup> and Fisher.<sup>9</sup> The more important recent publications are cited below.



received scant attention. The constituents involved in basic exchange are among the most labile and reactive components of soils. Nevertheless, current literature and standard texts make but brief reference to the relationship between the replaceable bases and such questions as the soil solution, soil acidity, the physical properties of soils, etc.

The vast majority of the former students of this subject have not determined the total content of the several replaceable bases nor their relative proportions. Most of the previous investigations have been made by bringing a salt solution to approximate equilibrium with the soil. Usually it is not possible to effect complete replacement in this way. A few investigators have subjected samples of soil to the leaching action of salt solutions. It is only comparatively recently, however, that serious effort has been made to determine the total content of the bases present in replaceable form, their relative proportions and their effects on the chemical and physical properties of the soil. The result has been that some very important relationships have passed largely unobserved.

As is well known the inference has been drawn that the capacity of soils to absorb potassium and ammonium bears some ill-defined relation to the clay content. Anderson *et al.*<sup>1</sup> have shown, however, that the absorptive power of the colloidal clay of different soils may vary widely. Our results show that the content of replaceable bases may bear but little relation to the total clay content, although the replaceable bases reside for the most part in the clay fraction of soils.

During the past ten years K. K. Gedroiz has discussed this subject at considerable length in a series of papers published in the Russian language.\*<sup>11, 12, 13, 14, 15, 16, 17</sup> These papers present by far the most illuminating discussion of the replaceable bases that has come to our attention. We shall make free reference to Dr. Gedroiz' views. In 1922 D. J. Hissink<sup>20</sup> also published an important paper on this subject based on a study of the soils of Holland.†

\* Within the past year English translations of these papers have been secured through the coöperation of Mr. C. S. Scofield of the U. S. Department of Agriculture. The translations were made by Dr. S. A. Waksman of the New Jersey Experiment Station.

† This paper was first published in Dutch in 1920 (Verslag Landbouwk. Onderzoek. Rijkslandbouwproefsta. 24: 144-248). It was republished in German in 1922 in a journal more generally accessible to Americans. An English summary of this paper, prepared by F. H. Smith, was published in 1923,<sup>21</sup> but the serious student will find it helpful to consult the full text. It is noteworthy that Dr. Hissink made no reference to Gedroiz' papers, although practically all the important points which Hissink discussed, with the exception of the electric-double-layer theory, had been considered by Gedroiz previously.

As is well known the amount of the different bases that may be displaced from a soil is related to the concentration of the salt solution used. Gedroiz<sup>12</sup> showed that between certain limits of concentration this is true not only under equilibrium conditions but also when the soluble products of the reaction are continually removed. By repeated extractions with 0.2 N solution of NaCl, KCl or  $\text{NH}_4\text{Cl}$  he found that the replacement was only partial, while normal solutions of any of the common salts will effect complete replacement. However, the rate at which salts of different bases bring about the replacement increases with the atomic weight and valency of the base. Sodium is less energetic in its effect than potassium, while magnesium is more active than potassium but less so than calcium and still less than aluminum and iron. Ammonium and potassium salts are similar in their replacing effect.

When the total content of the several bases present in replaceable form has been substituted by some other base, or when all of the replaceable bases except one have been replaced by a salt of that base, the soil is said to be saturated with respect to that base. Since the replacement reactions are reversible this latter base may in turn be substituted by any other base. These facts are extremely useful in studying this subject. By taking advantage of them it is possible to secure an insight into the significance of the replaceable bases and to determine the total content, inter-relationships and influences of these bases on the various properties of soils.

As will be more fully discussed below it is not entirely certain as yet whether the replaceable bases are chemically combined as true silicates, or whether they are held in some sort of adsorption complex. Nevertheless, it is important to understand that that part of the several bases which is present in replaceable form is quite definite in a given soil and is distinguishable from the remaining portion of the same bases. In fact, soils may contain certain bases in at least four forms. In addition to the replaceable forms, the bases may occur as, (1) water-soluble salts such as chloride, nitrate and sulphate, (2) carbonates and (3) the crystalline silicates and phosphates such as orthoclase, plagioclase, biotite, hornblende, apatite and many other readily distinguishable silicates. The problem dealt with here involves, therefore, the determination of the replaceable bases in the presence of one or more of the other forms.



Our previous investigations have been conducted with special reference to alkali soils. The present paper will be devoted to a consideration of the total content of the several bases present in replaceable form in (1) normal soils of neutral or alkaline reaction, (2) alkali soils, (3) acid soils. A brief discussion of the theoretical aspects of the replacement phenomenon will also be given. In this work we have made free use of the suggestions offered by Gedroiz and Hissink and with the exception of minor points our results have fully confirmed their conclusions.

## I. METHODS

Early in this work it became apparent that  $\text{NH}_4\text{Cl}$  is especially well adapted to the determination of the replaceable bases. The extracts obtained with solutions of this salt may be analyzed with comparatively little difficulty and it has the additional advantage of making possible a simple means of distinguishing between solubility effects and true replacement.

Since the replacement reactions involve an interchange of bases between the soil and the solution, a chemical equivalent of  $\text{NH}_4$  must remain in the soil for every cation brought into solution by replacement. Hence the amount of  $\text{NH}_4$  that is absorbed by the soil is a measure of the total cations replaced. When expressed as chemical equivalents the difference between the absorbed  $\text{NH}_4$  and the total bases in the extract is due for the most part to the solution of soil minerals. With the important exception of soils which contain calcium or magnesium carbonates, we have found no soil in which this difference is of very great magnitude, although solubility effects apparently always affect the results to some extent. The bases originally present in the soil as water-soluble salts, which in alkali soils may be considerable in amounts, must, of course, be subtracted from the amounts found.

That solubility effects as contrasted with replacement have some influence on the results is shown by the fact that the sum of the bases found, expressed as chemical equivalents, usually amounts to a somewhat greater quantity than that represented by the  $\text{NH}_4$  which the soil absorbs. The same conclusion is also indicated by the presence of more or less  $\text{SiO}_2$  in the extract. The extract obtained from every neutral or alkaline soil which we have examined has been found to



contain appreciable amounts of  $\text{SiO}_2$ , amounting in some cases to as much as 0.10 per cent of the soil. This seems to be equally true whether the replacing salt be  $\text{NaCl}$ ,  $\text{KCl}$  or  $\text{NH}_4\text{Cl}$ . With soils of coarse type such as sandy soils the amount of bases that is merely dissolved rather than replaced, although small in absolute quantity, may nevertheless constitute a considerable percentage of the total bases found. This seems to be especially true of coarse soils of comparatively recent geological origin. For this reason heavy types of soil are best suited to a study of the replaceable bases. However, we have made the determination successfully with several sandy loam soils.

The method we have used is with minor modifications a combination of that recommended by Hissink<sup>20</sup> and one of those proposed by Gedroiz.<sup>15</sup> The procedure was as follows: Twenty-five gm. of air dried soil and 250 cc.  $\text{NH}_4\text{Cl}$  solution were placed in a flask, shaken by hand and then held over night in an oven kept at  $70^\circ \text{C}$ . The following morning the contents of the flask were thrown on a folded filter. After the solution had drained through the filter the soil remaining in the flask was transferred to the filter and the residue was leached with successive portions of  $\text{NH}_4\text{Cl}$  solutions until 1000 cc. of filtrate was obtained.

Eight hundred cc. of the filtrate was transferred to a large porcelain dish and concentrated to a small volume on a water bath. Fifty to 75 cc. strong  $\text{HNO}_3$  was then added and the evaporation continued to dryness. By this means the  $\text{NH}_4\text{Cl}$  was decomposed and a residue obtained consisting of nitrates of the bases extracted from the soil together with small amounts of  $\text{SiO}_2$ . The residue was brought to dryness two or three times after adding strong  $\text{HCl}$  in order to convert the nitrates into chlorides and dehydrate the silica. After removing the  $\text{SiO}_2$  by filtration, the bases were determined by the use of standard methods of chemical analysis.

The soil residue remaining after the extraction with  $\text{NH}_4\text{Cl}$  was leached with distilled water until free from  $\text{Cl}$ . It was then transferred to a flask, 400 cc.  $\text{H}_2\text{O}$  and 50 cc. strong  $\text{NaOH}$  solution were added and the  $\text{NH}_4$  determined by distillation.

All determinations were made in duplicate and certain of them were repeated several times. Closely concordant results were almost always obtained.

In studies on methods we used a clay loam soil of the Ramona series (431), on which considerable experimental data have already been published from this laboratory.<sup>7, 22</sup> The soil is almost exactly neutral, contains no carbonate and is practically free from water-soluble salts.

#### EFFECT OF CONCENTRATION OF $\text{NH}_4\text{Cl}$

The results reported in table 1 show that both the total amount of bases extracted and the  $\text{NH}_4$  absorbed increased with increasing concentrations of  $\text{NH}_4\text{Cl}$  up to a certain concentration above which the results were fairly constant. These data indicate that the soil contains fairly definite amounts of replaceable bases and that complete replacement requires the use of a concentration of  $\text{NH}_4\text{Cl}$  slightly more than one-half normal. With concentrations greater than normal the increased amount of the bases found, which was mainly calcium, seems to be due entirely to solubility effects. It is noteworthy that K and Na may be more readily replaced than the divalent bases and Mg somewhat more so than Ca. These results agree with those of Gedroiz and Hissink.

TABLE 1

EFFECT OF CONCENTRATION OF  $\text{NH}_4\text{Cl}$  ON THE DETERMINATION OF REPLACEABLE BASES

Concentration of solution	Per cent of soil				Milligram equivalents	
	Ca	Mg	K	Na	Total bases	$\text{NH}_4$ absorbed
N/32.....	0.240	0.055	0.048	0.013	18.4	17.0
N/16.....	0.315	0.061	0.050	0.017	22.8	21.3
N/8.....	0.375	0.069	0.039	0.018	26.2	24.2
N/4.....	0.408	0.072	0.039	0.019	28.2	26.2
N/2.....	0.423	0.073	0.041	0.021	29.1	27.1
N/1.....	0.436	0.073	0.053	0.028	30.4	27.3
2N.....	0.453	0.078	0.051	0.028	31.6	27.5
3N.....	0.453	0.076	0.051	0.027	31.4	27.4

As a check on the method for the determination of absorbed  $\text{NH}_4$  the soil residue was freed from  $\text{NH}_4\text{Cl}$  by leaching with 80 per cent alcohol. In other cases the  $\text{NH}_3$  was driven out of the soil by the use of an alkaline solution of normal  $\text{CaCl}_2$ . The results were quite similar



to those reported above. As further evidence on the accuracy of the method other portions of the soil were completely saturated with K and Ca by leaching with solutions of KCl or  $\text{CaCl}_2$ . The soil residue was then leached free from Cl and the replaceable K and Ca were determined by treatment with a normal solution of  $\text{NH}_4\text{Cl}$  as described above. The results expressed as milligram equivalents per 100 gm. soil were 27.1 for K and 27.2 for Ca, while the  $\text{NH}_4$  absorbed as determined by distillation was 27.3. Thus it is shown that the  $\text{NH}_3$  obtained by distillation gives an accurate measure of the total bases replaced. These data further show that the replacement takes place by chemical equivalents, whether K, Ca or  $\text{NH}_4$  salts are used.\*

### RATIO OF SOIL TO SOLUTION

The volume of solution with which it is necessary to leach a given quantity of soil in order to effect complete replacement was also studied. In each case 25 gm. of soil and 250 cc. normal  $\text{NH}_4\text{Cl}$  were held over night at  $70^\circ \text{C}$ . Duplicate portions were then leached with  $\text{NH}_4\text{Cl}$  solution until 500 cc., 1000 cc. and 2000 cc. of leachate were collected. The results (table 2) show that substantially the same amount of replacement took place in each case. As might be expected, however, solubility effects became more pronounced with the more prolonged leaching (compare the total base found with the  $\text{NH}_4$  absorbed).

TABLE 2  
RESULTS OBTAINED BY LEACHING WITH DIFFERENT VOLUMES OF NORMAL  
 $\text{NH}_4\text{Cl}$  SOLUTION

Volume of solution	Per cent of soil				Milligram equivalents	
	Ca	Mg	K	Na	Total bases	$\text{NH}_4$ absorbed
500 cc.....	0.415	0.070	0.043	0.026	28.7	27.5
1000 cc.....	0.436	0.073	0.053	0.028	30.4	27.3
2000 cc.....	0.456	0.075	0.053	0.032	31.8	27.4

\* Milligram equivalent signifies chemical equivalents expressed in milligrams and hence its determination involves a consideration of the atomic weight and valency. The data were obtained by dividing the milligrams of a given base found per 100 gm. of soil by its atomic weight and multiplying the quotient by the valency of the base. For example, 100 gm. of this soil when saturated with Ca contains 544 milligrams of replaceable Ca.  $\frac{544}{40} \times 2 = 27.2 \text{ M.E.}$



RATE OF REPLACEMENT

Gedroiz and Hissink have called attention to the remarkable speed of the replacement reactions. In their work on this point they employed equilibrium conditions, under which complete replacement does not usually take place. It seemed desirable to determine the effect of varying the time of digestion with  $\text{NH}_4\text{Cl}$  solution before filtering and leaching the residue. The experiments were made with normal  $\text{NH}_4\text{Cl}$  at room temperature instead of at  $70^\circ \text{C}$ . as before.

It will be observed (table 3) that contact between the soil and the  $\text{NH}_4\text{Cl}$  solution for a period of ten minutes, followed by the time necessary to secure 1000 cc. of filtrate, effected almost as great replacement as contact for 16 hours. It is probable that merely leaching this soil with no preliminary shaking will give approximately complete replacement. By referring to the preceding experiments it will be noted, however, that lower results were obtained at room temperature than at  $70^\circ \text{C}$ .

TABLE 3  
EFFECT OF TIME ON THE REPLACEMENT OF BASES

Time of contact	Per cent of soil				Milligram equivalents	
	Ca	Mg	K	Na	Total bases	$\text{NH}_4$ absorbed
10 minutes.....	0.372	0.060	0.030	0.013	24.9	23.4
30    "       .....	0.384	0.059	0.034	0.014	25.6	23.6
60    "       .....	0.386	0.062	0.032	0.016	25.9	23.1
3 hours.....	0.403	0.072	0.041	0.025	28.2	24.4
6    "       .....	0.397	0.064	0.050	0.019	27.2	24.2
16   "       .....	0.400	0.073	0.047	0.020	28.1	24.1

Since an important object of this work was to determine the total content of the replaceable bases, all the remaining data were obtained by first digesting 25 gm. of the soil with 250 cc. normal  $\text{NH}_4\text{Cl}$  solution for several hours at  $70^\circ \text{C}$ ., and then leaching to one liter. It is probable, however, that a more rapid method and one requiring less extravagant use of  $\text{NH}_4\text{Cl}$  will give accurate results.

### EFFECT OF $\text{CaCO}_3$

When calcium carbonate is present difficulties arise in the determination of the replaceable Ca for the reason that the carbonate is soluble to some extent in solutions of all the common salts and markedly soluble in  $\text{NH}_4\text{Cl}$  solutions. Hissink attempted to avoid this difficulty by extracting with two successive liters of normal  $\text{NaCl}$  solution. He considers the Ca found in the first liter minus that in the second to be a measure of the total replaceable Ca. The theoretical basis for this method rests on the assumption that as much  $\text{CaCO}_3$  will be dissolved by the first liter as by the second, but since replacement takes place extremely rapidly and since the solubility of  $\text{CaCO}_3$  is repressed by the presence of Ca ions in the solution the results obtained by Hissink's method may be a little low.

Gedroiz<sup>15</sup> recommended the determination of the total  $\text{CO}_2$  in the soil before and after extracting with  $\text{NH}_4\text{Cl}$  solution. From the data thus obtained he introduced a correction on the assumption that for each mol of  $\text{CO}_2$  removed from the soil one mol of  $\text{CaCO}_3$  was dissolved.

TABLE 4  
EFFECT OF  $\text{CaCO}_3$  ON THE DETERMINATION OF REPLACEABLE BASES

CaCO <sub>3</sub> added	Per cent of soil				Milligram equivalents	
	Ca*	Mg	K	Na	Total bases	NH <sub>4</sub> absorbed
None.....	0.436	0.073	0.053	0.028	30.4	27.3
0.5 per cent.....	0.436	0.073	0.053	0.028	30.4	28.0
1.0 " ".....	0.434	0.072	0.052	0.027	30.2	28.0
2.0 " ".....	0.392	0.071	0.053	0.028	28.1	28.0
4.0 " ".....	0.315	0.072	0.053	0.028	24.3	23.2

\* The Ca data were corrected for the  $\text{CaCO}_3$  dissolved by the  $\text{NH}_4\text{Cl}$  solution.

The data in table 4 show the results obtained where varying amounts of  $\text{CaCO}_3$  were added. The determinations were made in the manner already described, with the additional determination of  $\text{CO}_2$  in the soil after extracting with  $\text{NH}_4\text{Cl}$  solution. Corrections were made in the Ca determinations for the  $\text{CaCO}_3$  dissolved. Where the amount of  $\text{CaCO}_3$  present was not more than 1.0 per cent of the

soil the results were quite accurate, but with higher percentages not all of the replaceable Ca was obtained. In still other experiments we have found, as pointed out by Gedroiz, that it is necessary to continue the extraction until practically all of the  $\text{CaCO}_3$  was dissolved. Then by making a correction for  $\text{CaCO}_3$  the content of replaceable Ca can be calculated. It is true, however, that prolonged extraction with  $\text{NH}_4\text{Cl}$  tends to dissolve increased amounts of bases combined in non-replaceable forms. As yet we have not found an entirely satisfactory method for the determination of replaceable Ca in the presence of high percentages of  $\text{CaCO}_3$ .

Where  $\text{CaCO}_3$  and  $\text{MgCO}_3$  occur the difficulties are still greater. Fortunately soils which contain both of these carbonates are not common, although we have found one such case. The presence of insoluble carbonates does not interfere with the determination of replaceable K and Na.

## II. NORMAL SOILS OF NEUTRAL OR ALKALINE REACTION

The following soils were studied: sample 302, Yolo loam which contains 0.102 per cent  $\text{CaCO}_3$  taken from Santa Paula, California; 430, Placentia sandy loam free from carbonate, from the Citrus Experiment Station Farm, Riverside, California; 431, Ramona clay loam free from carbonate taken from La Habra, California; 529, Porterville clay loam which contains 1.395 per cent  $\text{CaCO}_3$  taken near Lindsay, California; 539, Chino clay loam with 0.904 per cent  $\text{CaCO}_3$  from Spadra, California; 2767, Yolo loam containing 0.250 per cent  $\text{CaCO}_3$  from Tustin, California; 6274, Olympic clay loam free from carbonate taken near Lemon Cove, California.

The data shown in table 5 have been corrected for water-soluble salts and  $\text{CaCO}_3$ . It will be seen that soil 430, the lightest type studied, contained by far the least amount of replaceable bases, and soil 302, the next lightest type, contained considerably less than the other samples. None of them contained more than traces of replaceable Al, Fe or Mn.



TABLE 5  
REPLACEABLE BASES IN NEUTRAL OR SLIGHTLY ALKALINE SOILS

Soil	Per cent of soil				Milligram equivalents		Relative proportion of bases			
	Ca	Mg	K	Na	Total bases	NH <sub>4</sub> absorbed	Ca	Mg	K	Na
430.....	0.083	0.016	0.017	0.021	6.7	4.6	61.2	19.4	6.0	13.4
431.....	0.436	0.073	0.053	0.028	30.4	27.3	71.7	20.1	4.3	3.9
302.....	0.250	0.051	0.046	0.045	19.8	14.4	63.1	21.2	6.1	9.6
520.....	0.655	0.136	0.034	0.049	47.0	*	69.6	24.0	1.9	4.5
539.....	0.635	0.176	0.037	0.062	49.9	44.1	63.5	29.3	1.8	5.4
2767.....	0.269	0.097	0.021	0.073	25.1	24.1	53.4	32.3	2.0	12.3
6274.....	0.401	0.127	0.039	0.043	33.5	28.5	59.7	31.6	3.0	5.7

\* Not determined.

When the results are expressed not as percentages of the soil but as percentages of the total replaceable bases, calculated as chemical equivalents, a different picture is presented. In every soil Ca comprised more than 50 per cent of the total bases found and Mg 20 or more per cent, whereas the replaceable K and Na were low, both absolutely and relatively. In each soil the sum of the divalent bases equalled 80 or more per cent of the total. These results are of special interest in connection with the alkali soils discussed below.

It is of interest to compare these California soils with the European soils reported by Gedroiz and Hissink.

	Relative percentage of replaceable bases			
	Ca	Mg	K	Na
California soils (av. 7 samples).....	63	25	4	8
Russian soils (Gedroiz, av. 2 samples).....	82	11	7	0
Holland soils (Hissink, av. 26 samples).....	79	13	2	6

It will be noted that the California soils contain relatively less replaceable Ca and greater amounts of replaceable Mg than the soils reported by Gedroiz and Hissink, but when the magnitude of the analytical error and the solubility effects are taken into consideration the relative proportions of K and Na are not greatly different. The total quantity of replaceable bases found in the several European soils differed quite widely. This was especially true with the two Russian

soils, one of which was a podsol and the other a tshernoziem, but they appear to be remarkably similar qualitatively.

The origin of the California soils is known fairly definitely, especially so in the case of soils 430, 431, 520, 2767 and 6274. Certain of them have been derived from granite, others from shales and sandstones which themselves were derived from granite and still others from hornblende gabbro. The origin of the European soils on the other hand is unknown to us; they probably came from mixed sources.

On the basis of these data there does not appear to be any fundamental difference in the California soils which can be traced to the minerals from which they were derived. These soils are probably much more recent geologically than those reported by Gedroiz and Hissink, a fact which may have some bearing on the variations noted in the ratio of replaceable Ca to Mg. Further reference will be made to this point later.

The importance of the fact that Ca and Mg comprise a very large percentage of the total replaceable bases in normal soils will become more evident when we consider alkali and acid soils.

### III. ALKALI SOILS

The following alkali soils have been investigated: sample 1869, Fresno fine sandy loam from the Kearney Vineyard, Fresno, California; 5190, Jordan fine sandy loam drawn near Salt Lake, Utah; 5696, Lahontan clay from Fallon, Nevada; 6145, silty loam from the University Farm, Tucson, Arizona; 6155, Hanford silty loam from Arlington, California. These soils contained relatively high concentrations of soluble salts either when sampled or within a comparatively recent period. The soluble materials consisted mainly of Na salts. In addition to NaCl and Na<sub>2</sub>SO<sub>4</sub> all of these samples except No. 6155 contained considerable Na<sub>2</sub>CO<sub>3</sub>. With the exception of soil 1869 substantial amounts of CaCO<sub>3</sub> were present, and soil 5190 also contained MgCO<sub>3</sub> or CaMg(CO<sub>3</sub>)<sub>2</sub>.

It will be observed (table 6) that the replaceable bases of these soils bear an altogether different relationship from that found in normal soils. Instead of Ca and Mg comprising a high percentage of the total, as in normal soils, all of these samples contained no replaceable Ca and with the exception of 6155 none of them contained replaceable Mg.



TABLE 6  
REPLACEABLE BASES IN ALKALI SOILS

Soil	Per cent of soil				Milligram equivalents		Relative proportion of bases			
	Ca	Mg	K	Na	Total bases	NH <sub>4</sub> absorbed	Ca	Mg	K	Na
1869.....	0	0	0.084	0.091	6.0	3.0	0	0	35.0	65.0
5190.....	0	0	0.143	0.137	9.5	7.0	0	0	39.6	60.4
5696.....	0	0	0.031	0.570	25.6	24.0	0	0	3.1	96.9
6145.....	0	0	0.072	0.174	9.4	5.2	0	0	19.1	80.9
6155.....	0	0.080	0.057	0.094	12.2	12.3	0	54.1	12.3	33.6

As stated above considerable  $\text{Na}_2\text{CO}_3$  was present in every sample except 6155, and the amount was sufficient to maintain a highly alkaline soil solution and thus precipitate as carbonates any Ca or Mg that may have been brought into solution by the replacing action of Na or otherwise. The soluble products of the reaction being thus removed from solution, the replacement of the divalent bases must have already proceeded to completion or approximately so. The presence of more or less  $\text{CaCO}_3$  in alkali soils is probably due in part at least to its formation through these reactions.

It should not be inferred from these data that all alkali soils are free from replaceable Ca. This will depend on the concentration of the soluble salts together with the presence or absence of alkali carbonates. If the concentration be comparatively low only a part of the replaceable Ca will be substituted by other bases. Undoubtedly alkali soils frequently occur which contain more or less replaceable Ca, but with the important exception of those cases where a considerable amount of soluble Ca salts occur, at least a part of the replaceable Ca must be substituted by other bases. The results are in harmony with Gedroiz' data on the saline soils of Russia and with those of Hissink on the polder soils of Holland.

In considering alkali soils it is important to bear in mind that the relative proportions of the different bases present in replaceable form at a given time may reflect, in some measure at least, the effects of soluble salts which were in contact with the soil at some period previous to the time of sampling as well as of those present when the sample is drawn. It is probable that the kind and amounts of soluble salts in a



given place vary from time to time owing to the vicissitudes of climate, the methods used in handling the soil, etc. At one period a high concentration may prevail and later this may be greatly reduced by the leaching action of rains and still later the composition of the salts may be changed as a result of the deposition of soluble salts from other places. The result will be that the relative proportions of the replaceable bases may fluctuate.

Soils 5190 and 5696 represent for the most part lacustrine materials which were deposited in saline water. The water finally drained away or evaporated and rains have leached out most of the salts, particularly in soil 5696. The result is that these soils now contain no replaceable Ca or Mg. The relatively high content of replaceable Mg and K in certain of the samples was probably brought about by the action of soluble K and Mg salts. High concentrations of salts of these bases occur at the present time in various places in western America.

As has been pointed out by several investigators the deflocculated condition which develops in many alkali soils upon leaching out the excess of soluble salts is one of their most pronounced characteristics. This condition frequently develops where little if any  $\text{Na}_2\text{CO}_3$  is present but is especially evident in black-alkali soils. A determination of the replaceable bases in samples drawn from certain so-called "slick spots" which are characterized by pronounced impermeability, has shown a high content of replaceable Na. Soil 5696 is an extreme example of this condition. This sample typifies a comparatively large area whose soluble-salt content is not exceptionally high at present, but upon which scarcely a vestige of plant life can be found. Fundamentally the toxicity of this soil is due to its high content of replaceable Na rather than to soluble salts as such. As will be shown in a later paper soils whose replaceable base consists mainly of Na may be extremely toxic.

The studies that have been made on this subject show quite clearly that the fundamental cause of the deflocculated condition in these and other alkali soils lies in the nature of the replaceable bases. Whenever Na constitutes any considerable percentage of the total replaceable bases the soil upon leaching out the excess of soluble salts is almost certain to manifest colloidal properties in high degree. It is important to understand, however, that it is not the absolute amount of replaceable Na but rather its relation to the replaceable divalent bases, Ca in

particular, that determines whether the soil will be excessively colloidal. We expect to develop this subject more fully in a separate paper.

In the practical treatment of alkali soils the soluble salts must, of course, be dealt with, but this is by no means the only consideration. Certain types of alkali soils will certainly not become normal or productive merely through removal of the excess of soluble salts as has so often been assumed in the literature of alkali soils and in reclamation practice. The replaceable Na must also be displaced before the soil can be said to be fully reclaimed. Unless this be done the efforts and expense devoted to drainage and leaching will, in some cases at least, avail little or nothing.\* As shown above the content of replaceable Na both absolute and relative varies widely in different soils. Other things being equal it seems safe to assert that the lower the content of replaceable Na the more readily may an alkali soil be reclaimed. In the present state of knowledge it seems doubtful whether a soil whose total content of replaceable Na is as high as that of soil 5696 can be reclaimed economically. The difficulty becomes still greater where both the soil and the subsoil contain a high content of replaceable Na.

Three of the above samples, Nos. 1869, 5190 and 6145, were taken from areas on which reclamation experiments are in progress. The results thus far obtained are by far the most successful in the case of soil 5190. Certain treatments applied to soil 6145 have also yielded encouraging results, while with soil 1869 almost identically the same kinds of treatments that have been used on 5190 and 6145 have yielded mediocre results. It will be noted that the replaceable bases are not greatly different in these soils, either quantitatively or qualitatively. These soils differ very greatly, however, in their content of  $\text{CaCO}_3$ . Soil 1869 contains a very low amount; soil 6145 a considerable amount and 5190 a very high amount. Our studies show that a given soil when saturated with Na becomes more permeable upon adding  $\text{CaCO}_3$  in excess. Calcium carbonate tends to be dissolved by the soil moisture, especially in the presence of decaying organic matter, and in consequence it promotes a gradual reversal of the replacement process, thus building up a system in which Ca has displaced the Na. If leaching conditions be maintained as has been the case with the practical experi-

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\* Scofield has recently published a method by which the combined water-soluble and replaceable Na may be determined.<sup>32</sup>



ments on soil 5190 the replaceable Na will give way to Ca, the former being leached out as  $\text{NaHCO}_3$  and in the course of time a soil system will result in which the replaceable bases will become normal. This view is also in harmony with the opinion of Gedroiz and Hissink.

Another important practical consideration is found in the composition of the irrigation water. That which has been used in the field experiments on soil 1869 contains about 50 parts per million total salts which are composed mainly of sodium compounds. On the other hand the water used to irrigate soil 5190 contains approximately 1000 p. p. m. in which the ratio of the monovalent to divalent bases is somewhat less than 2. The water applied to soil 6154 is intermediate between the other two both in concentration and composition.

Thus it appears that a high content of  $\text{CaCO}_3$  and an irrigation water relatively rich in Ca and Mg salts are favorable to the reclamation of alkali soils. In such cases a Na saturated soil may be comparatively easily converted into a soil with normal properties, whereas in the absence of one or both of these conditions the reclamation may be difficult.

#### ALKALI SOILS WHICH CONTAIN SOLUBLE Ca SALTS

Many alkali soils contain considerable amounts of soluble Ca salts in addition to Na salts. We have determined the replaceable bases in two such soils (table 7). Although these soils contained large amounts of soluble salts, a considerable part of which was Na compounds, the relative amounts of the replaceable bases were found to be similar to those of normal soils. The reason for this must be apparent from the preceding discussion. The soluble Ca salts were sufficiently high to prevent the replacing action of Na. Where the amount of soluble Ca in proportion to Na is sufficiently great Na is not able to replace other bases. With low total concentrations small amounts of Ca may be replaced when the ratio of Ca to Na is as 1:2; whereas with high concentrations the ratio of divalent to monovalent bases may be as 1:5 or more and still no replacement will take place.



TABLE 7  
REPLACEABLE BASES IN SOILS WHICH CONTAIN SOLUBLE SODIUM AND  
CALCIUM SALTS

Soil	Per cent of soil				Milligram equivalents		Relative proportion of bases			
	Ca	Mg	K	Na	Total bases	NH <sub>4</sub> absorbed	Ca	Mg	K	Na
6157.....	0.380	0.162	0.040	0.047	35.5	31.2	53.6	38.0	2.8	5.6
6158.....	0.333	0.074	0.014	0.019	23.8	22.5	69.7	25.7	1.3	3.3

Upon leaching out the excess of soluble salts these two soils became pulverent and manifested properties quite similar to those of normal soils. The extreme deflocculation referred to above was not manifest. Such soils are readily amenable to reclamation by simple drainage and flooding. A considerable portion of the alkali areas of California is probably of this nature.

#### IV. ACID SOILS

The following acid soils were studied: 3232, Clermont silt loam from North Vernon, Indiana; 6251, a clay loam of an unclassified series from Mendocino County, California; 6275, Rhonerville clay loam from Rhonerville, California; 6276, Hagerstown clay loam from the Pennsylvania Experiment Station; 6277, Melbourn clay loam from western Oregon; 6278, Greenville clay loam from South Carolina. The results are recorded in table 8.

The content of replaceable Ca, Mg, K and Na was extremely low in these soils as compared with that of the neutral or alkaline soils of similar texture. The average total amount of these bases in the acid soils was 4.1 milligram equivalents and in the non-acid soils 29.6 milligram equivalents. Each of the acid soils contained more or less replaceable Al and Mn and two of them replaceable Fe. When expressed on the basis of chemical equivalents the trivalent bases were found to comprise a considerable percentage of the total. In fact the sum of the trivalent bases was in excess of the combined monovalent and divalent bases in soil 6276 and was almost as great in soils 6275 and 6277. The NH<sub>4</sub> absorbed was approximately equal to the sum of all the bases found.

TABLE 8  
REPLACEABLE BASES IN ACID SOILS

Soil	Per cent of soil							Milligram equivalents								
	Ca	Mg	K	Na	Al	Fe	Mn	Ca	Mg	K	Na	Al	Fe	Mn	Total bases	NH <sub>4</sub> absorbed
3232.....	0.068	0.011	0.008	0.011	0.013	.....	0.011	3.4	0.8	0.2	0.5	1.4	.....	0.6	6.9	6.1
6251.....	.027	.010	.005	.010	.008	.....	.007	1.3	.8	.1	.4	.9	.....	.4	3.9	3.1
6275.....	.053	.024	.002	.008	.025	.....	.018	2.6	2.0	.....	.3	2.8	.....	1.0	8.7	8.6
6276.....	.021	.011	.009	.019	.010	.009	.049	1.0	.9	.2	.8	1.1	.5	2.7	7.2	6.8
6277.....	.037	.014	.007	.027	.003	.001	.047	1.8	1.1	.2	1.2	.3	.1	2.6	7.3	5.0
6278.....	.060	.012	.006	.020	.002	.....	.007	3.0	1.0	.1	.9	.2	.....	.4	5.6	3.2

On the basis of these data there appears to be a fundamental difference both qualitative and quantitative between the replaceable bases of acid and those of non-acid soils. It should not be inferred, however, that acid soils necessarily contain a low content of replaceable bases or replaceable Al, Fe or Mn.\* As will be shown in the following section of this paper, a neutral soil may become acid upon treatment with carbonated water. In this case only a small part of the bases need be substituted by hydrogen in order to make the soil distinctly acid.

It has long been known that an acid solution is obtained by shaking certain soils with a neutral solution of K or Na salts. The Hopkins method for the determination of soil acidity is based on this fact. Veitch<sup>39</sup> and others have explained this fact on the basis of replaceable trivalent bases. There is considerable difference of opinion, however, as to whether the acidity is due to the presence of hydrolyzable salts of the trivalent bases, or whether the trivalent bases are actually replaced by the salt. Comber has proposed a qualitative test for acid soils based on the amount of Fe brought into solution by KCNS<sup>5</sup> or potassium salicylate.<sup>6</sup> He holds that Fe is replaced by K of these salts. It does not follow, however, that the acidity of soils in general is due entirely to salts of Fe or to replaceable Fe. Our data show that the content of replaceable Fe is not always proportional to the total content of replaceable trivalent bases and that a soil may be decidedly acid without containing more than traces of replaceable Fe.

It may be pointed out that the benefits to be derived from the application of lime appear to be determined not solely by the pH value of the soil, but also by the composition, concentration and the buffer properties of the soil solution. These latter are determined by the solubility of constituents not involved in replacement as well as by the readiness with which the replaceable bases are brought into solution. It is probable, however, that the crop-producing power of any soil whose content of replaceable Ca is low and which does not contain  $\text{CaCO}_3$  will be increased by the application of lime. This is

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\* Since this manuscript was written we have examined four other acid soils sent to us from widely separated localities east of the Mississippi River. Each of these samples contains substantial amounts of replaceable Al, Fe and Mn. Their total content of replaceable bases is also relatively low. Two of them are quite similar to the acid soils reported herein both qualitatively and quantitatively. The others contain somewhat greater amounts of replaceable bases, but neither is strongly acidic.



especially true when legumes or other crops which have a high Ca requirement are grown. The constituents concerned in basic exchange are the most labile and reactive components of soils. They are probably important sources from which the soil solution derives its Ca content.

Gedroiz and Hissink have pointed out that favorable physical properties are promoted by a high content of replaceable Ca in proportion to the other replaceable cations. As will be shown later the physiological properties as determined by culture experiments may be profoundly modified by alterations in the normal ratio of these bases. When  $\text{CaCO}_3$  is absent a considerable amount of replaceable Ca seems to be one of the essentials for the sustained production of high yields of crops. The content of replaceable Ca, therefore, constitutes an important characteristic of soils.

Hissink showed that a part of the replaceable Ca can be easily removed from soils by extraction with carbonated water. He holds that the soil when so treated becomes unsaturated with bases, that is, hydrogen ions of the carbonated water displace the bases from the soil. He believes that soils tend towards this condition in humid climates. Many Holland soils are only partially saturated with bases. Hissink found that soils which contain similar amounts of clay may differ materially in their total content of replaceable bases. This fact is attributed to unsaturation in the sense just mentioned. He proposed a scheme for expressing the degree of saturation by designating the sum of the replaceable bases as (S) and the total capacity for bases as (T). The difference between (T) and (S) expressed as chemical equivalents represents the quantity of base required to bring the soil to complete saturation. Gedroiz believes that the processes involved in the formation of podsol soils likewise involve the substitution of hydrogen, derived from meteoric waters and decaying organic matter, in the place of more or less of the replaceable bases. He further holds that the substituted hydrogen can itself be replaced by bases, but only, however, with much greater difficulty than can the replaceable bases.

Bradfield<sup>2</sup> has shown that colloidal clays separated from different soils have the power to neutralize different amounts of dilute alkaline solutions. In other words they possess different degrees of acidity. He also presented strong evidence that  $\text{Al}_2\text{O}_3$  and  $\text{SiO}_2$  present in the colloidal material of an acid soil occur in chemical combination as

alumino silicate.<sup>3, 4</sup> It is possible that the colloidal alumino silicates of different soils may contain variable amounts of bases, and if so the acidity may be due to substituted hydrogen or unsaturation in the sense of Gedroiz and Hissink. Gile and his associates<sup>19</sup> have found that the colloidal clay materials of different soils have the power of absorbing H<sub>2</sub>O, NH<sub>3</sub> and malachite green in quite variable amounts. In the case of the dye the differences found range from 0.0584 gm. to 0.4128 gm. per gram of the colloid.

### ABSORPTION OF Ca(OH)<sub>2</sub>

Samples of the acid soils reported in table 8 were digested for several hours at 70° C. with 0.04N Ca(OH)<sub>2</sub> and then thoroughly leached with Ca(OH)<sub>2</sub> solution. They were then washed with distilled water in order to remove the excess of Ca(OH)<sub>2</sub>. The wash water at first became strongly alkaline showing that an excess of Ca(OH)<sub>2</sub> had been used, and in most cases it continued to be alkaline after prolonged leaching. After the alkalinity had been reduced to a low level and practically all of the water-soluble Ca had been removed, the soil was treated with normal NH<sub>4</sub>Cl exactly as was done originally in the determination of replaceable bases.

TABLE 9  
BASES EXTRACTED BY NH<sub>4</sub>Cl AFTER FIRST TREATING THE SOIL WITH Ca(OH)<sub>2</sub>

Soil	Per cent of soil						Milligram equivalents	
	Ca	Mg	K	Na	Al	Fe	Total bases	NH <sub>4</sub> absorbed
431.....	.958	.043	.044	.039	.....	.....	54.2	27.4
3232.....	.378	.012	.012	.029	*	*	21.4	6.9
6251.....	.199	.017	.011	.019	*	*	12.4	3.2
6275.....	.766	.011	.011	.016	.....	.....	40.2	16.7
6276.....	.564	.006	.004	.009	.009	.013	30.9	10.4
6277.....	.892	.007	.003	.015	.003	.004	47.1	20.7
6278.....	.437	.008	.002	.013	.003	.....	23.4	3.5

\* Not determined.

By comparing the data shown in tables 8 and 9 it will be observed that the treatment with Ca(OH)<sub>2</sub> greatly increased the amount of Ca that was capable of being brought into solution with NH<sub>4</sub>Cl. The Ca



thus absorbed is soluble in  $\text{NH}_4\text{Cl}$  solution, but not necessarily as a result of replacement of bases as indicated by the  $\text{NH}_4$  data. Similar results were obtained when the treated soil was extracted with  $\text{NaCl}$  instead of  $\text{NH}_4\text{Cl}$ .

• The replaceable base content of soils 6251 and 6278 both of which are heavy clays was still extremely low after treatment with  $\text{Ca}(\text{OH})_2$ . As yet we have found no evidence that their content of replaceable base can be increased by any simple treatment. After treatment with  $\text{Ca}(\text{OH})_2$  certain of these soils still contained Al and Fe soluble in  $\text{NH}_4\text{Cl}$ . It is of special interest that the total amount of replaceable bases, as measured by the amount of  $\text{NH}_4$  absorbed, was not increased in certain of the acid soils, while in others it was markedly increased. Soils 6275, 6276 and 6277 apparently contain replaceable hydrogen, whereas the other acid soils do not. With the former a part of the absorbed Ca apparently entered into replaceable form analogous to that normally present. In this case the Ca probably replaced hydrogen, but the capacity of soils to absorb  $\text{Ca}(\text{OH})_2$  is not limited by their content of replaceable hydrogen or other cations. For example, the neutral soil 431 discussed above has the power to absorb greater amounts of  $\text{Ca}(\text{OH})_2$  than any acid soil that we have studied. Other neutral soils are also able to absorb notable amounts of  $\text{Ca}(\text{OH})_2$ , but the absorbed Ca does not enter into replaceable form.

## V. SPECIAL EXPERIMENTS AND DISCUSSION

Gedroiz<sup>16, 17</sup> holds that the replaceable bases occur either as humates or in zeolitic forms, the latter predominating in mineral soils. He uses the term "zeolite" not in the strict sense of the mineralogist, but because of the similarity between the properties of mineral soils and true zeolites. He suggests three possible modes of formation and origin of the so-called zeolitic constituents: (1) comminution by mechanical agencies of the minerals from which the soil has been derived, (2) formation of secondary silicates as a result of the weathering of the primary minerals, (3) colloidal precipitation of  $\text{SiO}_2$  and  $\text{Al}_2\text{O}_3$  occasioned by the opposite charge which these colloids bear. The colloidal complex thus formed absorbs the bases which take part in replacement. While Gedroiz states that all three of these processes may be involved to some extent, he considers the last named to be the



most important. His idea of the nature of the union between the bases and the colloidal complex is not entirely clear, however, nor why it is that such a colloidal complex is better able to absorb bases than colloidal silica or other electro-negative colloids.

CLAY AND THE COARSE FRACTION OF SOILS

By the use of the method outlined by Anderson, *et. al.*,<sup>1</sup> we have separated a considerable quantity of the so-called ultra-clay from three normal soils of neutral or only slightly alkaline reaction. The replaceable bases were then determined in this material. The soils from which the clay was separated contained widely different amounts of clay material. As shown in table 10 the clays also differed materially in their content of replaceable bases, although the clay from each soil contained a much higher percentage than the soil from which it was separated (compare tables 5 and 10).

TABLE 10  
REPLACEABLE BASES IN THE CLAY AND COURSE MATERIALS OF SOILS

Materials	Per cent				Relative proportion of bases			
	Ca	Mg	K	Na	Ca	Mg	K	Na
430—Clay.....	0.502	0.100	0.065	0.062	66.6	22.0	4.2	7.2
431—Clay.....	.978	.209	.135	.033	66.4	26.3	5.1	2.1
520—Clay.....	1.104	.217	.069	.085	70.0	23.0	2.3	4.7
430 > 100 mesh.....	.024	.026	.032	.032	21.4	39.3	14.3	25.0
431 > 100 mesh.....	.070	.039	.049	.062	33.0	30.2	11.3	25.5
430 > 100 mesh ground < 200 mesh.....	.030	.026	.041	.045	22.4	32.8	14.9	29.9
431 > 100 mesh ground < 200 mesh.....	.089	.040	.053	.069	36.7	27.5	10.8	25.0

The part of two of these soils which would not pass a 100-mesh sieve was also studied both with and without grinding to pass a 200-mesh sieve. The grinding undoubtedly reduced a part of the coarse material to colloidal dimensions. It will be noted that the content of replaceable bases was quite low in both cases and that the grinding produced very little effect on replacement. A considerable part of the bases found was probably brought into solution as a result of solubility

effects rather than by replacement. It will be noted also that the relative proportions of the several bases extracted from the coarse fractions were quite different from those found in the clay materials. The coarse materials were composed very largely of feldspars, hornblende, mica, etc., the minerals from which these soils have been derived.

Other experiments were made in which the whole soil was ground fine enough to pass a 200-mesh sieve, but the grinding produced no appreciable effect on the replaceable bases.

These experiments show that the replaceable bases reside mainly in the finer fractions of these soils, probably in the clay, as has long been held to be the case. They indicate further that the bases have not become replaceable as a result of the mere comminution of the primary minerals.

TABLE 11  
REPLACEABLE BASES IN MATERIALS FROM WHICH SOILS HAVE BEEN DERIVED

	Per cent				Relative proportion of bases			
	Ca	Mg	K	Na	Ca	Mg	K	Na
Hornblende gabbro.....	0.095	0.018	0.009	0.004	71.2	22.7	3.0	3.0
Shale.....	.491	.090	.087	.058	66.8	20.4	6.0	6.8

In contrast to the preceding results we have found that the replaceable bases of a clay soil of the Olympic series occur in relative proportions quite similar to those of the finely ground hornblende gabbro (table 11) from which this soil has been derived. However, the total content of replaceable bases in the former was very much greater than in the latter. A sample of argillaceous shale free from CaCO<sub>3</sub> and ground to pass a 200-mesh sieve was also studied. Soil 539, reported in table 5, has been derived in part from the formation from which the sample of shale was obtained. The shale was relatively rich in replaceable bases and the bases were present in relative proportions quite similar to those of the soil nearby.

It was also found that heating a soil to 110° C. for a period of 30 hours produced no effect on the replaceable bases. These results are in harmony with Way's data on pipe clay and finely ground burnt clay pipes. They show that these materials contain substantial amounts of bases in replaceable form which are not necessarily converted into non-replaceable forms either by the consolidating processes involved in the formation of shale or by relatively high temperature.



A light-colored, slightly compacted, shale-like layer about one inch in thickness occurs about two feet below the surface where soil 539 was drawn. This material was free from  $\text{CaCO}_3$  but contained 1.58 per cent replaceable Ca. Gedroiz reported several clay soils from Russia with more than one per cent of replaceable Ca.

As stated above the replaceable bases of normal non-acid soils are composed mainly of Ca, with Mg present in considerable amount and with quite low percentages of monovalent bases. This fact was explained by Gedroiz on the basis of relative solubilities. During the process of formation and subsequently, the most soluble constituents would, of course, be most likely to pass out into the drainage. Another factor is found in the fact that insoluble  $\text{CaCO}_3$  is frequently laid down along with the other constituents. Regardless of the origin of the replaceable bases, the relative proportions of the different bases originally present, or whether they are components of definite chemical compounds or are adsorptively held, the gradual solution of  $\text{CaCO}_3$  will result in the substitution of Ca for the other replaceable bases. Since the monovalent bases are somewhat more easily replaced than Mg, the tendency is towards a low percentage of monovalent bases and a high percentage of Ca, with Mg somewhat intermediate.

Hissink has pointed out that this process has actually taken place to a demonstrable extent in certain polder soils of Holland. These soils soon after having been flooded by sea water less than a century ago contained considerably less replaceable Ca and correspondingly more Na than at present, but they have sustained considerable loss of  $\text{CaCO}_3$  since these areas were first reclaimed. It seems probable that  $\text{CaCO}_3$ , brought into solution as the bicarbonate, has displaced Na from its position in the replaceable complex. We believe that similar reactions may be promoted in the reclamation of alkali soils, with the consequent improvement of such soils.

#### EFFECT OF DILUTE ACIDS

Gedroiz<sup>18</sup> claims that when a soil is treated with dilute acid, the bases are replaced by hydrogen. We have made a brief study of this point, using water saturated with  $\text{CO}_2$  and also three strengths of HCl. Twenty-five-gram portions of soil 431 were shaken with the solutions at room temperature and leached with the solutions until one liter of



the HCl extract and four liters of the carbonic-acid extract were obtained. Each liter of the latter was analyzed separately. It was found that the first liter contained much the greatest amount of Ca, but the last also contained appreciable amounts. The total amounts of bases dissolved are recorded in table 12.

TABLE 12  
EFFECTS OF DILUTE ACIDS AS COMPARED WITH NH<sub>4</sub>Cl

Solution used	Per cent of soil							
	Ca	Mg	K	Na	Al	Fe	Mn	SiO <sub>2</sub>
H <sub>2</sub> O saturated with CO <sub>2</sub>	0.105	0.030	0.014	**				0.089
0.01N HCl.....	.283	.040	.031	.017	.081		.003	.060
0.02N HCl.....	.336	.052	.033	.026	.103		.015	.081
0.04N HCl.....	.440	.070	.055	.031	.152*	**	**	.140
1.0N NH <sub>4</sub> Cl.....	.436	.073	.053	.028				.054

\* Precipitate contained some iron.  
\*\* Not determined.

As the strength of the acid was increased the amount of bases dissolved increased. With the 0.04N HCl almost exactly the same amounts of Ca, Mg, K and Na were found as were replaced from the original soil by normal NH<sub>4</sub>Cl, but considerable Al, Fe and Mn were dissolved also.

The soil residues from the preceding experiment were leached with water until free from Cl. They were then extracted with normal NH<sub>4</sub>Cl exactly as in the preceding replacement studies. The results recorded in table 13 show that the soil previously extracted with dilute acid still contained considerable amounts of replaceable bases. As stated already this soil originally contained only the merest traces of trivalent bases soluble in NH<sub>4</sub>Cl. Treatment with dilute HCl so affected the soil, however, as to render the Al, Fe and Mn distinctly replaceable by NH<sub>4</sub>Cl. Carbonic acid on the other hand had no effect on the replaceability of these constituents. The solubility of SiO<sub>2</sub> was also materially increased. The amount of NH<sub>4</sub> absorbed from NH<sub>4</sub>Cl was reduced somewhat but not greatly by the preliminary treatment with acid (compare tables 5 and 13).

TABLE 13  
CONSTITUENTS DISSOLVED BY NH<sub>4</sub>Cl AFTER EXTRACTION WITH DILUTE ACID

Soil previously extracted with	Per cent of soil							Milligram equivalents							NH <sub>4</sub> ab- sorbed		
	Ca	Mg	K	Na	Al	Fe	Mn	SiO <sub>2</sub>	Ca	Mg	K	Na	Al	Fe		Mn	Total bases
H <sub>2</sub> CO <sub>3</sub> .....	.342	0.058	0.028	.016	.....	.....	.....	*	17.1	4.8	0.7	0.7	.....	.....	.....	23.3	25.4
0.01N HCl.....	.197	.011	.019	.014	.057	.007	.005	.134	9.8	.9	.5	.6	.6	.4	.3	18.8	23.3
0.02N HCl.....	.161	.017	.024	.014	.074	.012	.009	.161	8.0	1.4	.6	.6	.8	.7	.5	20.0	22.8
0.04N HCl.....	.081	.051	.029	.036	.088	.047	*	.177	4.0	4.2	.7	1.6	9.8	2.6	.....	22.9	23.9

\* Not determined.

If treatment with dilute acids merely brings about a substitution of hydrogen for the several bases present in replaceable form, then the amount of the bases dissolved by the acid plus that replaced by  $\text{NH}_4\text{Cl}$  after the acid treatment should approximate the amounts of replaceable bases originally present. The data recorded in table 14 show that this was the case when carbonic acid was used, but not so with dilute  $\text{HCl}$ . These determinations indicate that dilute  $\text{HCl}$  attacks not only the constituents normally involved in replacement reactions but still others as well. When the strength of the acid is sufficient to dissolve quantities of bases similar to those which are replaceable by neutral salt solutions, it seems that rather deep-seated chemical reactions take place which are not involved in replacement with salts.

TABLE 14  
TOTAL AMOUNTS OF CONSTITUENTS EXTRACTED BY ACIDS AND  $\text{NH}_4\text{Cl}$

	Per cent of soil							
	Ca	Mg	K	Na	Al	Fe	Mn	SiO <sub>2</sub>
NH <sub>4</sub> Cl alone.....	0.436	0.073	0.053	0.028	.....	.....	.....	0.054
H <sub>2</sub> CO <sub>3</sub> and NH <sub>4</sub> Cl.....	.447	.088	.042	*	.....	.....	.....	.089
0.01N HCl and NH <sub>4</sub> Cl..	.480	.051	.050	.031	.138	.007	.008	.194
0.02N HCl and NH <sub>4</sub> Cl..	.497	.069	.057	.040	.177	.012	.024	.242
0.04N HCl and NH <sub>4</sub> Cl..	.521	.121	.084	.067	.240	.047	*	.317

\* Not determined.

It is difficult to say whether the trivalent bases reported in table 13 were really brought into solution as a result of replacement or by solubility effects. The fact that the total amount of bases found, including Al, Fe and Mn, when expressed as chemical equivalents, was only slightly less than the  $\text{NH}_4$  absorbed suggests that these bases were actually replaced, but it is possible, although hardly probable, that  $\text{NH}_4$  replaced hydrogen thus producing a solution sufficiently acid to effect the solution of these bases. A thorough understanding of the reactions which take place when soils are treated with dilute acids might throw important light on the origin of replaceable Al, Fe and Mn in acid soils.\*

\* After this paper had gone to press our attention was called to the investigations of Daikuhara (Bul. Imp. Cent. Agr. Exp. Sta., Japan, 2: 1-40, 1914) and Liesegang and Kappen (Landw. Vers. Sta., 99: 191-230, 1921) in which the subject of soil acidity was discussed from the standpoint of replacement. The latter showed that the acidity of KCl extracts of certain naturally acid soils and of soils that had been treated with dilute acids was due entirely to replaceable Al. The mechanism by which Al becomes replaceable upon treating a neutral soil with dilute acids was also discussed.



Extraction with dilute HCl, however, does not necessarily affect the capacity of the soil to hold replaceable bases as is shown by the following experiment. The same soil was first extracted with 0.02N HCl, then leached free from Cl. The residue was treated with  $\text{Ca}(\text{OH})_2$  solution and again leached with water until practically free from soluble Ca. The soil was then treated with normal  $\text{NH}_4\text{Cl}$  solution as in the preceding determination of replaceable bases. It was found that the total content of replaceable bases as measured by the  $\text{NH}_4$  absorbed was thus restored to its original amount.

The above data suggest that certain soils are acidic not merely because they contain replaceable hydrogen in the sense employed in this paper but because of the presence of soluble trivalent bases which form hydrolyzable salts or possibly because of the presence of silicic acids. The application of lime to such soil would be expected to lower the solubility of these bases and at the same time augment the supply of Ca that is readily soluble in carbonated water.

It is not possible at present to explain the fact that soils of similar type vary so greatly in replaceable base content. There is some evidence that, other things being equal, the extent to which the soil materials have been weathered and the amount of leaching the soil has undergone determine the content of replaceable bases. Meteoric waters undoubtedly tend to displace these bases but this would hardly lower the total content very materially as long as  $\text{CaCO}_3$  is present. When the carbonate has been exhausted the replaceable bases probably gradually disappear. It is well known that  $\text{CaCO}_3$  frequently occurs in considerable amount in the substratum a foot or more below the surface, while the surface soil may be free from  $\text{CaCO}_3$ . This is especially true in semi-arid regions and often where there is no evidence that  $\text{CaCO}_3$  was an original constituent of the soil. It is probable that the carbonate has been derived from the replaceable Ca.

### ADSORPTION

Our results agree with those of Gedrioz and Hissink in showing that the rate at which the replacement takes place is extremely rapid. Hissink holds that this is evidence that the replaceable bases occur on the surface of the soil particles. Since the reaction between salt solutions and soils involves an interchange of bases with little or no change in the anions of the soil or the solution, Hissink believes, in

common with many other workers, that the bases are present in a state of adsorption. Much of the published literature on this subject is characterized, however, by vagueness. Frequently there is but little indication as to the meaning intended to be conveyed by the term adsorption. It seems to have been employed by various writers on soils more as a convenient word than to express a definite idea.

Hissink hypothecates that certain anions, possibly  $\text{SiO}_3$  and organic radicles, and the cations involved in replacement form a sort of electric double layer around the soil particles. The anions bearing a negative charge are considered to be on the interior and the cations with their positive charge on the exterior of this double layer. Hence an exchange of cations takes place between the soil and the solution without any effect on the anions. This view suggests the well known electric-double-layer theory of Helmholtz and the applications that have been made of it by various students of cataphoresis and adsorption phenomena. This view differs from the Helmholtz theory, however, in that the double layer is regarded by Hissink as an essential part of the soil particles themselves, whereas in the application of the Helmholtz theory to other systems the disperse phase and the dispersion medium are both usually considered as contributing to the formation of the electric double layer. This latter assumption seems to be necessary to account for the fact that certain colloids are electro-negative when dispersed in one medium and electro-positive in another medium.

Although it is true that various crystalline silicates are capable of undergoing replacement reactions as shown by Lemberg,<sup>25</sup> Sullivan,<sup>34</sup> Cummins and Kelley<sup>7</sup> and others, it seems safe to say that with the soils we have studied and possibly with soils generally the bases have become replaceable mainly as a result of metamorphosis occasioned by the weathering process. Whether the bases occur in ordinary chemical union with silica as alumino silicates, or are held in a state of adsorption, can not now be definitely stated.

As shown in the preceding section of this paper, soils both acid and neutral have the power to absorb substantial amounts of  $\text{Ca}(\text{OH})_2$  without exchange of cations. When thus absorbed  $\text{Ca}(\text{OH})_2$  appears to be loosely held. It slowly passes into solution with water, yielding an alkaline solution. Replaceable Ca, on the other hand, is practically insoluble in water. Mattson<sup>27</sup> explained the fact that clay and certain other colloids absorb  $\text{Ca}(\text{OH})_2$  on the assumption that these materials



first take up the OH ions by adsorption. This increases the negative charge of the particles and promotes the adsorption of the positively charged Ca ions. Vernadsky<sup>40</sup> has recently pointed out that numerous aluminosilicates, including the zeolites, feldspars, etc., have a common aluminosilicate nucleus, and that these silicates form a series of addition compounds with  $\text{Ca}(\text{OH})_2$ . He also claims that other compounds of aluminum and silicon, not belonging to the aluminosilicate class, also form addition compounds with  $\text{Ca}(\text{OH})_2$ .

The results of recent investigations in physical chemistry indicate that there is no sharp distinction between ordinary chemical union and adsorption processes. Some prominent workers in this field look upon the process of adsorption which finely divided materials, clay in particular, manifest in striking degree as being essentially chemical. Langmuir<sup>24</sup> presented evidence which supports this view. He considers the forces involved in adsorption to be analogous to those taking part in the union of molecules in crystal formation. X-ray examination of various crystals has indicated that the atoms are not combined as in the gaseous form of the compound. The ordinary valency appears to be supplanted by what has been designated as residual or secondary valency. Recently Svedberg<sup>35</sup> has stated that Langmuir's theory offers the best explanation yet advanced for the adsorption of ions by colloids. The formation of the addition compounds of silicates and  $\text{Ca}(\text{OH})_2$  just referred to and the absorption of  $\text{Ca}(\text{OH})_2$  by soils might be similarly explained.

G. N. Lewis<sup>26</sup> has undertaken to show the mechanism of this process on the basis of the electronic concept. In his view the ordinary chemical bond is a pair of electrons shared mutually by two atoms. He suggests that crystals are built up by the sharing of pairs of electrons which occur on the surface of molecules and that adsorption implies the sharing of previously unshared pairs of electrons between the substances involved. He points out that the well known adsorptive power of  $\text{SiO}_2$  is in harmony with this theory. Still others claim that adsorption of ions or compounds from solutions involves hydrolysis. Quite recently Miller<sup>27</sup> has shown that carbon absorbs bases from solutions of electrolytes as a result of hydrolysis.

The tendency, therefore, is to bring adsorptive processes into line with chemical principles.



There is much evidence that the taking up of  $\text{Na}_2\text{CO}_3$  and soluble phosphates by soils and the absorption of dyes,<sup>12</sup> which have long been explained on the basis of adsorption, are essentially chemical processes involving the formation of insoluble compounds. For example, soil 431 has the power to absorb notable amounts of  $\text{Na}_2\text{CO}_3$ , but after the replaceable Ca and Mg have been substituted by Na this soil no longer has this power. Bradfield<sup>3</sup> has shown that the action of dilute alkali on colloidal clay obeys definite chemical laws. The reaction is in harmony with the assumption that the colloid contains a weak acid of low solubility. In his opinion it is unnecessary to take recourse to adsorption concepts in order to explain soil acidity, as has been done by numerous writers on the subject. Truog,<sup>36</sup> Sharp and Hoagland<sup>33</sup> and others hold similar views.

The experimental data presented above indicate that the replaceable bases are chemically combined in soils. Some recent data suggest that the content of replaceable bases is one of the fundamental characteristics of soils. While much still remains to be determined, we believe that the replaceable bases occur as chemical compounds, probably as complex alumino silicates which have been formed as a result of weathering. There seems no good reason why such compounds may not be formed, and certainly the assumption of such vaguely defined forces as are commonly understood by the term adsorption does not help to clarify a subject which by its very nature is extremely complex.

From this point of view these constituents may be looked upon as being similar to the zeolites, as has long been held to be the case. They represent a transition stage between the igneous minerals and insoluble oxides or kaolin but can not be identified petrographically because of the small size of the particles. Strong support for this view is afforded by the mode of occurrence and formation of the crystalline zeolites in the state of nature. The reactive constituents of soils are not necessarily zeolitic, however, for certain nonzeolitic minerals undergo basic exchange with salt solutions.

Recently Ganssen (Gans)<sup>10</sup> has discussed the composition of the zeolitic constituents of soils on the basis of the ratio of  $\text{SiO}_2 : \text{Al}_2\text{O}_3 :$  bases. The analyses upon which his discussion rests were made by acid digestion. Gedroiz<sup>13</sup> has shown that acids of the strengths used in making these analyses dissolve substantial amounts of bases which do not undergo exchange reactions. Moreover, no method has yet been

discovered by which the complex which is involved in the replacement phenomenon can be decomposed into its components without decomposing other constituents as well. By separating the colloidal material from the soil by mechanical means and then studying its composition and properties, important light may be thrown on the composition of the constituents involved in replacement reactions.

As stated before the replacement reactions appear to take place almost instantaneously. They do not go to completion at equal rates throughout, however. Gedroiz has repeatedly emphasized that it requires prolonged extraction in order to effect the replacement of the last traces of Ca, but that the replaceable Mg, K and Na may be more readily displaced. He was unable to effect complete replacement with 0.2 normal solutions of salts even when the soluble products were removed by repeated decantation. However, partial replacement took place at once with this and even less concentrated solutions. Hissink also found that a lesser amount of leaching with salt solutions is required to remove the replaceable monovalent bases than the divalent bases. Our experience is in agreement with theirs. It is difficult to harmonize these facts with the adsorption hypothesis of Hissink. If every atom of a given base is located on the exposed surface of particles, as he assumes, one should be as readily displaced as another.

The fact that a part of the replaceable Ca, and possibly of other bases as well, is more easily replaced than other parts suggests that more than one chemical compound is involved. Considering the great variation in the composition of natural silicates, including the true zeolites, the probable variation in the structural arrangement within the various molecules of silicates, and the numerous possibilities for chemical combinations between the several silicic acids and the different bases, it is almost certain that a varied assortment of degradation products is formed during the course of weathering. The differences in the rate of replacement might also be due in part at least to the occurrence of molecular aggregates of the replaceable compounds, sub-microscopic crystals in fact, some of whose chemically combined bases occur on the interior of the particle and can be replaced only as a result of diffusion.

It should not be inferred from the preceding discussion that organic substances are not involved in the phenomenon of replacement. On the contrary there is much evidence that a part of the replaceable



bases usually occurs in some sort of combination with organic matter. These constituents probably exert important influences on soils. It has seemed logical in our treatment of this subject, however, to limit the present discussion to the inorganic constituents.

### SUMMARY

1. By taking advantage of the exchange reaction which takes place between soils and  $\text{NH}_4\text{Cl}$  the total content of the several bases present in replaceable form may be readily determined. In order to effect complete replacement it is necessary either to extract a given sample of soil many times with the salt solution, or else subject it to leaching conditions. The latter is the more practicable.

2. The exchange of bases proceeds to completion only when a sufficiently concentrated solution is used. With  $\text{NH}_4\text{Cl}$  this concentration is approximately normal. Since the exchange takes place stoichiometrically, the amount of  $\text{NH}_4$  absorbed by the soil is chemically equivalent to the sum of all the bases replaced. The absorbed  $\text{NH}_4$  may be determined by first leaching out the excess of  $\text{NH}_4\text{Cl}$  and then distilling the residue with a solution of  $\text{NaOH}$  or some other alkaline solution of sufficient concentration.

3. The difference between the absorbed  $\text{NH}_4$  and the sum of the several bases extracted represents solubility effects rather than replacement. This difference is not great except with soils which contain considerable amounts of soluble salts or insoluble carbonates. The amount of  $\text{CaCO}_3$  dissolved by  $\text{NH}_4\text{Cl}$  may be calculated from  $\text{CO}_2$  determinations made before and after the extraction.

4. The replaceable bases of several neutral or slightly alkaline soils from California are composed mainly of Ca (50 or more per cent of the total replaceable bases); Mg is present in the next highest amount (20 to 30 per cent), but there are only small amounts of K and Na.

5. Alkali soils are characterized by a relatively large amount of replaceable Na and a correspondingly low amount of replaceable Ca. Several black-alkali soils were studied which contain no replaceable Ca. The relative relationship between the several replaceable bases of alkali soils depends on the composition and concentration of the soluble salts. Where the soluble salts contain sufficient amounts of Ca compounds, the ratio of the replaceable bases may be similar to that of



normal soils. Replaceable Na must be reckoned with in the practical treatment of alkali soils.

6. The acid soils examined are characterized by a low total content of replaceable bases, and by the presence in replaceable form of more or less trivalent bases (Al, Fe or Mn). The amount of these latter bases may constitute 50 or more per cent of the total replaceable bases.

7. Acid soils have the power to absorb substantial amounts of  $\text{Ca}(\text{OH})_2$ . A part of the absorbed  $\text{Ca}(\text{OH})_2$  may enter into replaceable form, but a much larger part may not. The absorption of  $\text{Ca}(\text{OH})_2$  is not confined to acid soils, however. A neutral soil was studied which absorbed large amounts of  $\text{Ca}(\text{OH})_2$  without an exchange of cations.

8. The greater part of the replaceable bases reside in the clay fraction of soils, but different clays vary greatly in their total content.

9. The hydrogen of carbonic acid may be substituted for a part of the replaceable bases. Dilute HCl likewise displaces the bases, but may attack other constituents as well. A neutral soil, which originally contained mere traces of trivalent bases in replaceable form, was found to contain substantial amounts after treatment with dilute HCl.

10. The replaceable bases are considered to be present not in a state of physical adsorption but as chemical compounds, probably as complex alumino silicates which have been formed as a result of weathering.

11. The results obtained in this investigation are in close agreement with those published by Gedroiz and Hissink.

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NUTRIENT AND TOXIC EFFECTS OF CERTAIN IONS  
ON CITRUS AND WALNUT TREES WITH ESPECIAL  
REFERENCE TO THE CONCENTRATION AND  
P<sub>H</sub> OF THE MEDIUM

BY

H. S. REED AND A. R. C. HAAS

UNIVERSITY OF CALIFORNIA PRINTING OFFICE  
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INTRODUCTION

The continuation of studies which have been presented in publications from this laboratory<sup>17, 18, 19</sup> has led to the results presented in this paper which should be regarded as a report of work in progress.

The absorption of ions and their distribution in the plant are not determined by such relatively simple factors as the concentration or the solubility of the substance in question, but are profoundly influenced by the coördinating activities of the living plant. An ion may have a specific effect upon a single organ and have little or no direct effect upon others. It is therefore important to study the activity of the plant in conjunction with its chemical analysis, if we are to appreciate the equilibrium existing between the plant and its surroundings. The farmer is obviously interested in maintaining an equilibrium suited to the proper physiological functioning of the plant. The nutrient or toxic effects of certain of the compounds to be discussed are problems of agriculture in semi-arid regions where irrigation is practiced. The present studies are intended to extend our knowledge of the effect of certain factors upon the growth and composition of

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\* Paper No. 110, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

young citrus and walnut trees. The present conditions of experimentation in this field of investigation are such that it is necessary to employ young seedling plants for some of the work. It is hoped that the investigation may be extended to include experiments on the absorption process in older trees.

## EXPERIMENTAL DATA AND DISCUSSION

### I. THE EFFECT OF SODIUM SULFATE ON WALNUT SEEDLINGS

#### *(Juglans regia)*

The following experiments were designed to show the effects of sodium sulfate added to a well-balanced nutrient solution (Hoagland's). The seedlings were grown two months in 40-liter water cultures in the glasshouse. Twelve uniform walnut seedlings whose radicles were from 6 to 10 cm. long were suspended in each culture through holes in paraffined wooden covers which supported the young plants. Two jars were used for each concentration of the culture solution. The solutions were changed at the end of the first month, more frequent changes being considered unnecessary since the concentrations were high and the volume of the solution per plant large. The volume of the solutions was maintained by frequent additions of distilled water. The illustrations show that walnut seedlings may be successfully grown in a nutrient solution by this means.

The seedlings of series A (table 1) grew in Hoagland's nutrient solution, which has the following composition expressed as parts per million:

Na	K	Ca	Mg	Fe	Mn	NO <sub>3</sub>	Cl	SO <sub>4</sub>	PO <sub>4</sub>	Total
7	185	159	54	1	0.1	718	10	216	105	1455.1

Series B, C, D, and E contained this solution plus 1500, 3000, 4500, and 6000 p.p.m. sodium sulfate, respectively. Since the highest total concentration was approximately 7500 p.p.m., we employed another series (series F) which contained the same salts as series A, but in five-fold concentration.

The seedlings made very satisfactory growth in series A, B, and F (fig. 1). In the higher concentrations the growth of tops and roots was restricted, and the leaf margins were dead like those shown in





fig. 2. The roots were less profusely branched and were dark colored. Growth of the lateral rootlets soon stopped and their apical portions were swollen like those shown in fig. 3. The seedlings of series F showed no abnormality although the initial concentration was 7275 p.p.m.; they differed from those grown in series A chiefly in the smaller growth of epicotyls and leaves.

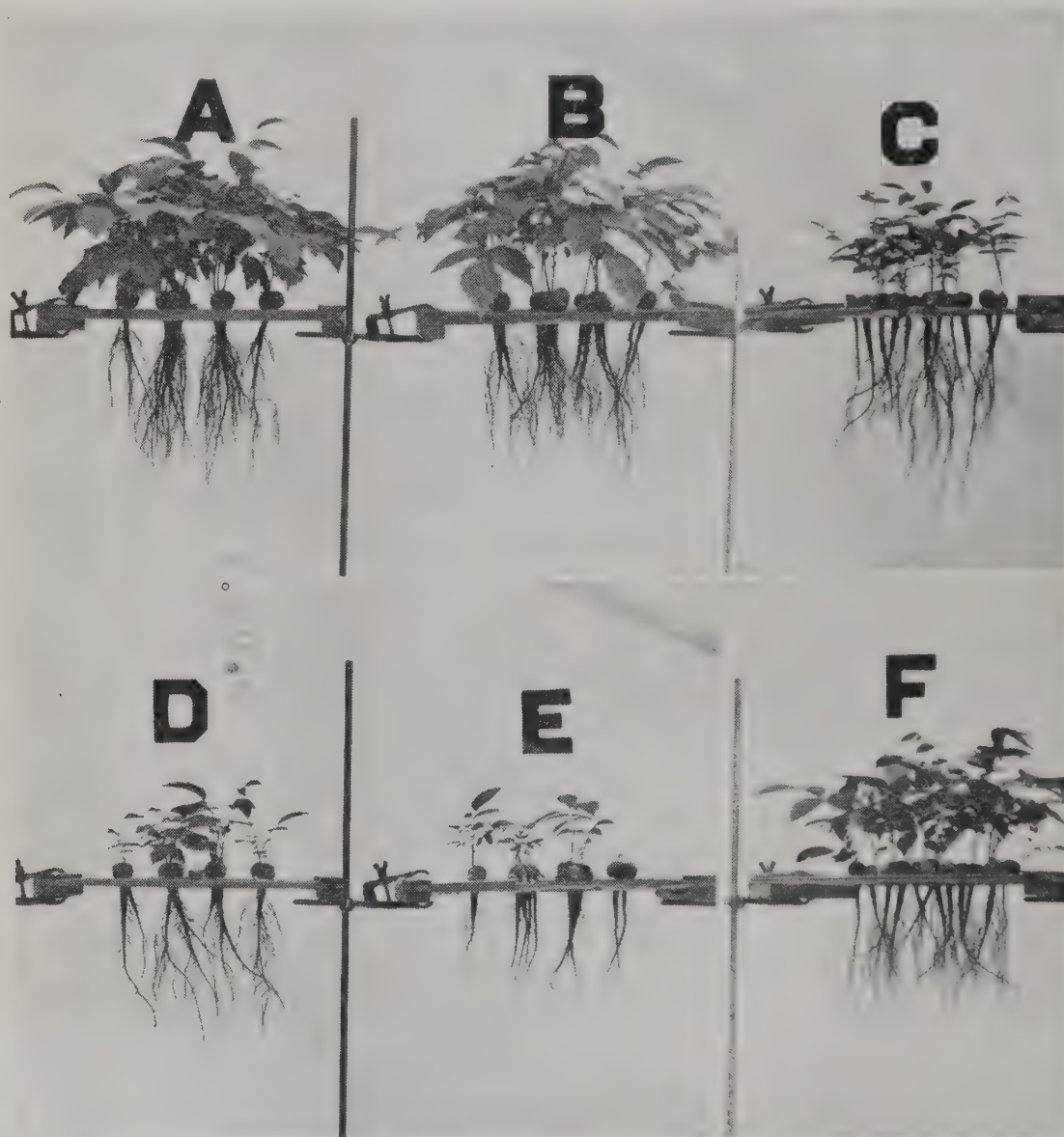


Fig. 1. Walnut seedlings which had grown two months in nutrient solutions containing sodium sulfate. A, in single-strength nutrient solution; B, C, D, E, in single-strength nutrient solution plus 1500, 3000, 4500, and 6000 p.p.m. sodium sulfate respectively; F, in nutrient solution having five times the ordinary concentration.

The fresh weight of the tops and roots of the plants (without cotyledons) in each series is given in table 1. It will be noted that growth of tops was more retarded by the higher concentrations of  $\text{Na}_2\text{SO}_4$  than that of roots, in fact the epicotyls entirely failed to

develop in some plants in the higher concentrations. The maximum ash content was found in the plants grown in series F.

The sulfur content of the plants, whether measured by the non-volatile  $\text{SO}_4$  or as total sulfur in the dry matter, reflected in a general way the amount of sulfate in the culture solution. There appears to



Fig. 2. Walnut leaf from plant grown in culture solution containing 4500 p.p.m. sodium sulfate. The dead margins were characteristic of the plants grown in the higher concentrations of sodium sulfate.



Fig. 3. Walnut root from culture solution containing more than 4000 p.p.m. sodium sulfate.

have been an increase in both the inorganic and organic compounds of sulfur.

The analyses of the ash show that active absorption of certain ions took place. The percentages of Na and of  $\text{SO}_4$  reflected the influence of these ions in the culture solutions up to a certain limit, beyond which an increase in the concentration had no effect.



The ash of the roots contained a higher per cent of Na than the tops with the exception of plants in series F. Where the culture solution contained 500 or more p.p.m. Na the per cent of this ion found in the ash of roots was nearly constant.

The ash of the tops contained a higher percentage of K than that of the roots. Although the percentage of K in the tops was fairly uniform in all series, the percentage of K in the roots was lower when the concentrations of  $\text{Na}_2\text{SO}_4$  in the solutions were higher.

The percentages of Ca and Mg although small, show a rather striking relation to the amounts of  $\text{Na}_2\text{SO}_4$  in the culture solution. The higher concentrations of  $\text{Na}_2\text{SO}_4$  seem to have diminished the absorption of Ca and Mg.

The roots were richer in  $\text{SO}_4$  than the tops, except in series F. Series C, D, and E were rather uniform in size (cf. fig. 1) and contained considerably higher amounts of  $\text{SO}_4$  (series A, B, and F). With the data at hand it is not possible to relate the growth entirely to the amounts of Na or of  $\text{SO}_4$  found in the plants. We are inclined to believe that the retarded growth in series C, D, and E is correlated to some extent with the lower percentages of Ca absorbed by the plants. In view of the conspicuous changes in the size and shape of lateral roots of the injured plants it is not improbable that their ability to absorb ions was modified because of altered arrangement and structure of the cells. The microscopical structure of the malformed roots awaits study.

## II. THE EFFECT OF SODIUM NITRATE ON WALNUT SEEDLINGS

Walnut seedlings were grown from April 11 to June 5 as water cultures in 2-quart glass jars, with one seedling in each jar. Each series contained 12 jars. The initial concentration of Na and  $\text{NO}_3$  is given in table 2. The solutions were renewed every two or three weeks.

With solution G (table 2) as a basis the other solutions were made by adding 1324, 2647, 3971, and 5295 parts per million of  $\text{NaNO}_3$ , respectively. The total concentrations are given near the top of table 2.

The growth of the walnut seedlings was similar to that in the preceding experiment, although the roots were less affected by the higher concentrations of  $\text{NaNO}_3$ . The greatest effect was noted in



TABLE 2

GROWTH AND COMPOSITION OF WALNUT SEEDLINGS IN NUTRIENT SOLUTIONS CONTAINING VARIOUS CONCENTRATIONS OF SODIUM NITRATE

Series	G		H		I		J		K	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Parts per million Na in culture solution.....	718 1455	7	366 1683 2780	724 2648 4102	1083	1442	36	71	31	90
Parts per million NO <sub>3</sub> in culture solution.....		718								
Parts per million total solutes in culture solution.....		1455								
Fresh weight per 10 plants (grams).....	77	92	72	90	46	75	36	71	31	90
Constituents of the dry matter (per cent):										
Ash.....	4.88	3.46	5.25	3.95	5.64	4.16	5.98	4.82	7.32	5.28
Total N.....	2.68	3.31	3.16	3.63	3.54	4.17	3.80	4.66	3.76	4.73
Constituents of the ash (per cent):										
Na.....	6.49	7.92	12.88	15.76	16.53	16.47	18.78	22.65	21.01	21.43
K.....	26.78	25.86	27.68	19.05	26.80	15.75	21.07	18.07	19.18	17.53
Ca.....	8.09	2.92	6.17	1.45	3.21	0.99	3.32	0.78	2.39	1.18
Mg.....	4.20	2.38	3.67	1.08	2.56	1.18	2.60	1.18	1.84	1.56
SO <sub>4</sub> .....	2.57	3.74	2.98	5.26	2.88	7.08	3.90	8.75	3.10	8.18
PO <sub>4</sub> .....	19.92	54.95	18.78	48.90	19.66	50.68	21.40	46.17	18.27	42.71

the tops of the seedlings. The leaves of plants in series I, J, and K showed dead margins somewhat as illustrated in fig. 2. The injury was increasingly severe in the higher concentrations of  $\text{NaNO}_3$ . Since the seedlings in solution F (table 1) showed no dead leaf margins when the total concentration of salts was 7275 p.p.m., it is improbable that the injury was caused solely by concentration. The root systems were well developed and healthy in appearance. The size of the plants is indicated by the fresh weights. It is interesting to note that the growth in series H was as good as in G, although the former solution contained over 1600 p.p.m.  $\text{NO}_3$ . The inhibiting effects of larger concentrations of  $\text{NaNO}_3$  appear to be shown mainly by the epicotyls.

The percentages of ash and of nitrogen were increased by the higher concentrations of  $\text{NaNO}_3$  although not proportionally to the concentration.

The percentage of Na in the tops and roots increased up to a certain point, beyond which no significant increase was found. The percentages of Ca and of Mg were substantially reduced by the more concentrated solutions.

In another experiment, walnut seedlings were grown two months in Hoagland's nutrient solution which contained  $\text{NaNO}_3$  or  $\text{Na}_2\text{SO}_4$  in concentrations of  $\frac{\text{M}}{46}$ . The solutions were renewed every two or three weeks. Duplicate cultures were made in stone jars holding 40 liters with 13 seedlings in each jar.

Both series made fairly good growth, though the leaves in the  $\text{Na}_2\text{SO}_4$  series showed some dead margins. The roots in both sets developed normally. The general appearance of the seedlings at the end of two months is shown in fig. 4. The fresh weight of 10 plants from the  $\text{NaNO}_3$  series was 194 g. and that of 10 plants from the  $\text{Na}_2\text{SO}_4$  series was 132 g.

The interpretation of this experiment is complicated by the fact that to one solution twice as much Na was added as to the other, but with these equi-molecular solutions the growth was better in the  $\text{NaNO}_3$  set.

The influence of the cation on walnut seedlings was further studied by an experiment consisting of three series of cultures. The first contained Hoagland's nutrient solution in four times the usual concentration; the second, Hoagland's nutrient solution (ordinary strength) plus 2692 p.p.m.  $\text{NO}_3$  as  $\text{Ca}(\text{NO}_3)_2$ ; the third, Hoagland's nutrient

solution (ordinary strength) plus 2692 p.p.m.  $\text{NO}_3$  as  $\text{NaNO}_3$ . The seedlings were grown for six weeks and the fresh weights of 10 plants were 150 g., 152 g., and 84 g., respectively.

These results indicate that, in these high concentrations Na was much less favorable to growth than Ca, and that  $\text{SO}_4$  is more toxic than equivalent concentrations of  $\text{NO}_3$ .



Fig. 4. Walnut seedlings grown two months in nutrient solutions plus equi-molecular concentrations of sodium salts.

N  $\frac{\text{M}}{46}$  sodium nitrate; S,  $\frac{\text{M}}{46}$  sodium sulfate.



TABLE 3  
EFFECT OF DIFFERENT CONCENTRATIONS OF CULTURE SOLUTION CONTAINING 500 PARTS PER MILLION NaCl, UPON  
GROWTH AND COMPOSITION OF ORANGE SEEDLINGS

Series	L	M	N	O
	Culture solution	Culture solution plus 500 p.p.m. NaCl	Double-strength culture solution plus 500 p.p.m. NaCl	Triple-strength culture solution plus 500 p.p.m. NaCl
Total concentration of solutes (p.p.m.).....	1455	1954	3408	4862
Fresh weights of 100 plants (grams).....	Tops    Roots 227    160	Tops    Roots 169    113	Tops    Roots 236    136	Tops    Roots 192    108
Dry weights of 100 plants (grams).....	72    33	50    25	66    29	57    25
Per cent of ash in the dry matter.....	10.40	11.94	12.39	13.32
Na (per cent of ash).....	5.22	13.13	11.57	9.78
Cl (per cent of ash).....	.75	9.52	7.94	6.86

TABLE 4  
COMPARATIVE EFFECTS OF NaCl AND CaCl<sub>2</sub> UPON GROWTH AND ABSORPTION BY WALNUT SEEDLINGS

Series	P		Q		R		S		T	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
P.p.m. Cl added to the culture solution.....		1542		1542		1542		1542		
P.p.m. Na added to the culture solution.....		1000		0		1000		0		
P.p.m. Ca added to the culture solution.....		0		871		0		871		
Concentration of nutrient solution (p.p.m.).....		1455		1455		7275		7275		5820
Total concentration of solutes (p.p.m.).....		3997		3868		9817		9688		5820
Fresh weights per 10 plants (grams) .....										
Per cent of ash in the dry matter.....	7.66	4.80	9.51	5.50	9.62	6.54	9.27	7.32	9.66	8.10
Constituents of the ash (per cent):										
Na.....	15.16	18.94	5.70	7.21	13.73	9.14	5.76	3.28	8.19	6.48
K.....	26.68	17.05	25.20	22.88	30.83	20.05	26.30	17.93	30.83	19.59
Ca.....	4.66	1.20	12.64	4.96	3.60	2.70	7.39	5.77	6.51	9.04
Mg.....	3.09	1.42	2.34	2.13	2.59	3.10	2.55	2.52	3.61	1.75
Cl.....	10.17	9.23	12.87	10.10	9.30	6.01	9.29	3.86	1.24	1.60
SO <sub>4</sub> .....	3.41	2.91	2.33	3.48	5.31	4.13	5.13	5.40	4.34	6.82
PO <sub>4</sub> .....	23.13	47.52	15.45	39.82	21.01	50.34	20.81	49.00	2.65	5.10

### III. THE ABSORPTION OF SODIUM AND CHLORIN FROM NUTRIENT SOLUTIONS OF DIFFERENT CONCENTRATION

It has been known for a long time that within a certain range there is some sort of relation between the concentration of ions in a solution and the amount absorbed. The data given above confirm the earlier results on this particular point. We wish to present some data which show that the absorption of an ion is affected by the concentration of other ions. In this instance 500 parts per million NaCl were added respectively to several concentrations of standard nutrient solution.

The experiments were made with St. Michael orange seedlings. Series L (table 3) contained Hoagland's nutrient solution during both periods of the experiment. Series M, N, and O contained Hoagland's solution plus 500 p.p.m. NaCl for 158 days (August 14 to January 19) and then the cultures were grown 33 days in solutions having increased concentrations of nutrient solution plus 500 p.p.m. NaCl. When the experiment was terminated no serious toxic effects were observed in any of the plants, although there were appreciable differences in the sizes of the plants.

Growth was most retarded in series M where the NaCl was added to single-strength nutrient solution. In series N the growth was practically as good as in series L which had no NaCl, which may indicate that the higher concentration of nutrient ions decreased the toxicity of the NaCl. In series O the plants were about the same as in series M, this fact possibly indicating that the concentration of solutes had in itself an inhibiting influence on growth. The percentage of ash in the dry matter progressively increased with increasing concentration of the solution. The percentages of Na and of Cl in the ash were greatest in series M with a progressive decrease in N and O. It would appear that there was something like a dilution of the NaCl due to the higher concentration of nutrient ions.

We may conclude therefore that (within certain limits of concentration) it is not alone the absolute amount of an ion that determines the amount that will be absorbed, but that its relation to the concentration of the other ions is also of considerable importance.



#### IV. THE CHLORIN CONTENT OF PLANTS GROWN IN SOLUTIONS OF DIFFERENT CHLORIDS

##### *a. Walnut seedlings.*

The question of the influence of cation upon the absorption of anion has been frequently raised in these and other experiments. Plate<sup>16</sup> observed that the growth of oat seedlings in gram-molecular solutions of chlorids of the different monovalent cations was by no means equal. The growth of tops in the various chlorids formed a descending series as follows:  $\text{NH}_4 > \text{K} > \text{Na} > \text{Rb} > \text{Li} > \text{Cs}$ . The growth of roots formed a similar series except that the positions of K and Na were reversed. Hoagland<sup>4</sup> found that there were no significant differences in the amounts of Cl absorbed from different salt solutions.

For the next experiment a concentration of 1542 p.p.m. Cl was used in conjunction with the standard nutrient solution (series P and Q, table 4) and with a five-fold concentration of the nutrient solution (series R and S). The control (series T) consisted of a four-fold concentration of nutrient solution. The figures at the top of table 4 show the initial amounts of the ions and the total concentration of the culture solutions. Four series of 26 walnut seedlings each were grown in 40-liter jars, from September 19 to October 30, the solutions being renewed every two to three weeks.

The seedlings in the first four series showed the toxic effects of the chlorids (fig. 5). The plants in series P (NaCl) were characterized by small leaves and a restricted growth of lateral roots, while those in cultures to which  $\text{CaCl}_2$  was added (series Q) produced good leaves and tops. The primary roots in the NaCl cultures were brown, while those in the  $\text{CaCl}_2$  cultures were white and thickened. The lateral roots in both series showed restricted growth (fig. 5), and those in series P (NaCl) were short and brown. The roots grew well in series Q ( $\text{CaCl}_2$ ) for a time, but decay set in at the upper end of the laterals near the primary root and eventually extended down to the tips. Growth in the more concentrated solutions (R and S) was more limited than in the other two series. The plants in the R series (NaCl) produced short epicotyls and small leaves. In the S series ( $\text{CaCl}_2$ ) the epicotyls were longer and the leaves were somewhat larger, but many leaves showed the type of "burning" which is characteristic of high salt concentrations.

Certain relations between absorption and composition of culture solution are shown in table 4. Attention is directed to the fairly constant ratio of chlorin to ash constituents in the four series. Where growth was approximately equal, there was practically the same absorption of Cl without regard to the amount of Na or Ca present. Plants in the concentrated solutions absorbed somewhat less Cl than those in the dilute solutions, probably as a result of the introduction of additional ions.



Fig. 5. Comparative effects of sodium chlorid and calcium chlorid on the growth of walnut seedlings. P, 1542 p.p.m. Cl as sodium chlorid plus single-strength nutrient solution; Q, 1542 p.p.m. Cl as calcium chlorid plus single-strength nutrient solution; R, 1542 p.p.m. Cl as sodium chlorid plus concentrated nutrient solution; S, 1542 p.p.m. Cl as calcium chlorid plus concentrated nutrient solution (cf. table 4).

The results of these series of cultures show that the toxic effects of NaCl and CaCl<sub>2</sub> on walnut seedlings, while similar, are not identical. In series P and Q, where the total concentration was not detrimental, NaCl appeared to check the growth of the tops more than CaCl<sub>2</sub>. In series R and S where the total concentrations were high, there was less difference in the effects of the two salts.

Another experiment with walnut seedlings was made to compare the effects of NaCl and KCl. Three series of cultures were made in 40-liter jars. The first received the nutrient solution in three times the ordinary concentration; the second and third received 1542 p.p.m.



Cl as KCl and NaCl respectively (table 5). The cultures were started December 27 and ended February 7 and the solutions were renewed every two or three weeks.

The growth of the seedlings in the NaCl series was somewhat better than that made in cultures conducted during the summer or early fall. The tops in the NaCl series showed no tendency towards burning like that formerly observed and the growth of roots was not so greatly retarded, although the development of lateral roots was conspicuously inferior to that of the control series (fig. 6).



Fig. 6. Comparative effects of sodium chlorid and of potassium chlorid on the growth of walnut seedlings. U, nutrient solution having three times the ordinary concentration; V, 1542 p.p.m. Cl as potassium chlorid plus nutrient solution; W, 1542 p.p.m. Cl as sodium chlorid plus nutrient solution.

TABLE 5  
GROWTH OF WALNUT SEEDLINGS IN NUTRIENT SOLUTIONS CONTAINING EQUIVALENT CONCENTRATIONS OF Cl FURNISHED AS NaCl OR AS KCl.

Series	U		V		W	
P.p.m. Cl added to the culture solution.....			1542		1542	
P.p.m. Na added to the culture solution.....					1000	
P.p.m. K added to the culture solution.....			1700			
Concentration of nutrient solution (p.p.m.).....	4365		1455		1455	
Total concentration of solutes (p.p.m.)....	4365		4697		3997	
Fresh weight per 10 plants (grams) .....	Tops	Roots	Tops	Roots	Tops	Roots
	52	110	81	104	40	90



The leaves in the KCl series, although large and broad, were pale green and eventually became yellow. The roots, though well branched, stopped growing and began to die before the end of the experiment. The injury to the lateral roots, like that previously noted in toxic concentrations of  $\text{CaCl}_2$ , was first noticed in the region next to the primary root, from which it extended toward the apex. The plants in the control series were healthy during the entire period of the experiment and produced splendid root systems.

The quantitative relations of the three series are shown by the fresh weights of the plants given in table 5. The weight of roots showed no significant differences, but the weights of tops were quite different. The figures indicate a distinct toxic action of the NaCl and a pronounced increase in growth in the KCl series. The increase in the latter was made during the first part of the experimental period; subsequently, the toxic effects gradually appeared. The condition is somewhat similar to that seen when equivalent amounts of  $\text{CaCl}_2$  were employed in former experiments.

There is an obvious difference in this case between the toxic effects of NaCl and KCl when equivalent amounts of Cl are used. It might be concluded that an early toxic effect of Cl was more or less prevented by the K ions. It is obvious that there is a time factor involved in the case of toxicity. The toxic effect of  $\text{CaCl}_2$  appears later than that of NaCl, but it may eventually terminate growth of the plant.

#### *b. Citrus seedlings.*

A series of cultures of rough-lemon seedlings was grown for 32 days in a complete nutrient solution with the addition of sodium or calcium chlorid. Eighteen jars each containing three seedlings were used for each concentration. The controls were grown in nutrient solution; the second set was grown in nutrient solution plus 308, 617, 1234 or 1851 p.p.m. chlorin, as sodium chlorid; the third set of plants was grown in solutions the same as the second except that the chlorin was added as calcium chlorid.

The seedlings grown in solutions containing 308 p.p.m. sodium chlorid showed no injury, either to roots or tops; those in solutions containing 617 p.p.m. showed a small amount of injury to the older leaves. The symptoms of injury were a recurved condition and death of the tissues at the margin of the leaf. When the concentration was greater than 617 p.p.m. chlorin, the amount of injury was greater. There was an appreciable, although small, stimulation to growth in all

TABLE 6  
GROWTH AND COMPOSITION OF WALNUT SEEDLINGS IN RELATION TO EQUIVALENT AMOUNTS OF VARIOUS ANIONS FURNISHED WITH SODIUM

Series	AA		BB		CC		DD		EE	
	Tops 44	Roots 40	Tops 41	Roots 63	Tops 20	Roots 54	Tops 42	Roots 52	Tops 82	Roots 68
P.p.m. Na in culture solution.....	1000	2692 NO <sub>3</sub>	1000	1542 Cl	1000	2649 HCO <sub>3</sub>	1000	2086 SO <sub>4</sub>	28	
P.p.m. of anion added.....	1455	5147	1455	3997	1455	5104	1455	4541	5820	5820
Concentration of nutrient solution (p.p.m.).....										
Total concentration of solutes (p.p.m.).....										
Fresh weight per 10 plants (grams).....										
Per cent of ash in the dry matter.....	8.26	6.24	8.28	5.96	6.59	4.88	7.66	6.30	9.66	8.10
Constituents of the ash (per cent):										
Na.....	17.25	23.10	17.38	21.38	25.34	24.54	15.24	25.41	8.19	6.48
K.....	24.41	12.34	26.90	11.86	28.18	11.39	29.28	7.73	30.83	19.59
Ca.....	5.57	2.83	3.34	1.87	1.46	2.61	3.83	1.72	6.51	9.04
Mg.....	3.40	1.24	2.32	.97	2.07	1.35	2.91	.91	3.61	1.75
Cl.....	1.04	2.29	13.22	14.48	.92	1.84	.98	1.86	1.24	1.60
SO <sub>4</sub> .....	2.74	9.18	3.06	3.91	4.32	9.73	9.52	16.89	4.34	6.82
PO <sub>4</sub> .....	8.83	11.55	9.30	12.71	21.16	11.15	5.73	8.15	2.65	5.10



of the jars which contained 2000 p.p.m. sodium chlorid. Equivalent concentrations of chlorin as calcium chlorid were somewhat less toxic to the plants. The nutrient solution employed contained 159 p.p.m. calcium, yet the addition of calcium chlorid appears to have had a distinctly beneficial effect upon the growth of the plants during the short period of the experiment. Valencia orange trees grown for a longer time in sand cultures receiving 1770 p.p.m. chlorin as calcium chlorid, have shown severe tip burn of the leaves with their subsequent abscission.

We have previously shown<sup>18</sup> that orange trees made poor growth in sand cultures when furnished 1000 p.p.m. NaCl, and that growth was much better when also 440 p.p.m. CaCl<sub>2</sub> were added. The difference should be referred to the fact that the first lot suffered, not only from the toxic action of NaCl, but also from the lack of Ca. The introduction of CaCl<sub>2</sub> improved conditions for growth in spite of the fact that it increased the concentration of Cl ions. So long as the concentration of Cl is not too high, the presence of Ca may tend to offset the injurious effect of Cl by promoting growth and hence diluting the concentration of Cl in the plant, but in higher concentrations of Cl, or with longer time, injury ultimately appears.

## V. THE EFFECT OF DIFFERENT SALTS OF SODIUM

We have discussed the effect of equal concentrations of Cl as NaCl and as CaCl<sub>2</sub>, and shall now consider the toxic effects of equal concentrations of Na when added in the form of NaNO<sub>3</sub>, NaCl, NaHCO<sub>3</sub>, or Na<sub>2</sub>SO<sub>4</sub>. Five series of walnut seedling cultures were prepared according to the plan shown at the top of table 6. Culture EE contained Hoagland's nutrient solution of four times the usual concentration and may be regarded as the control. Each series contained 26 walnut seedlings, which were grown from July 17 to September 1. It will be seen that each of the four toxic solutions contained 1000 p.p.m. Na. Previous experiments had shown that this concentration of Na salts is decidedly toxic to walnut seedlings. The control solution had only 28 p.p.m. of Na, but its total concentration of solutes was 5800 p.p.m. at the outset. In spite of this high concentration there was no evidence of harmful effect.

The general appearance of the plants from the different solutions at the end of the experiment is shown in fig. 7, and the fresh weights of tops and roots are shown by diagram 1.



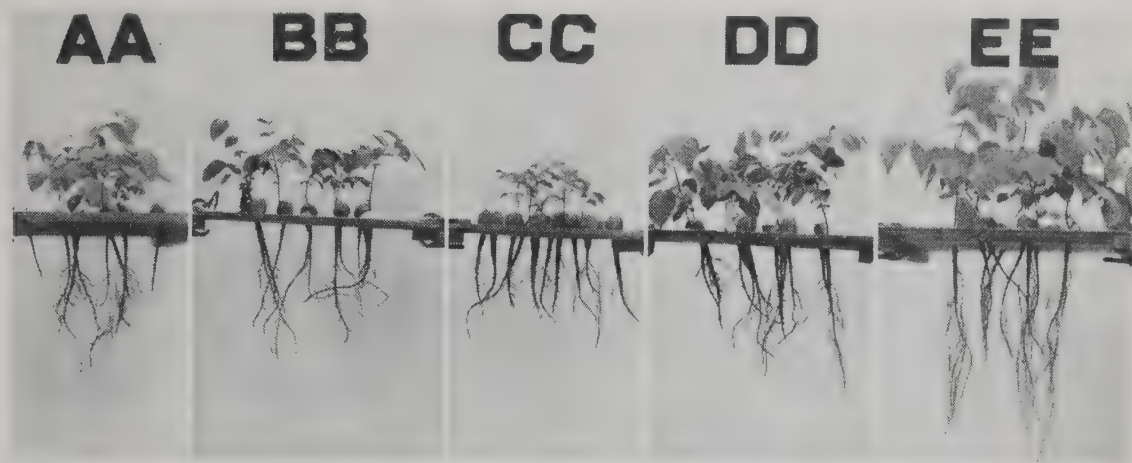


Fig. 7. Walnut seedlings showing effects of 1000 p.p.m. sodium in combination with different anions. AA, sodium nitrate; BB, sodium chlorid; CC, sodium bicarbonate; DD, sodium sulfate; EE, nutrient solution having four times the ordinary concentration (cf. table 6).

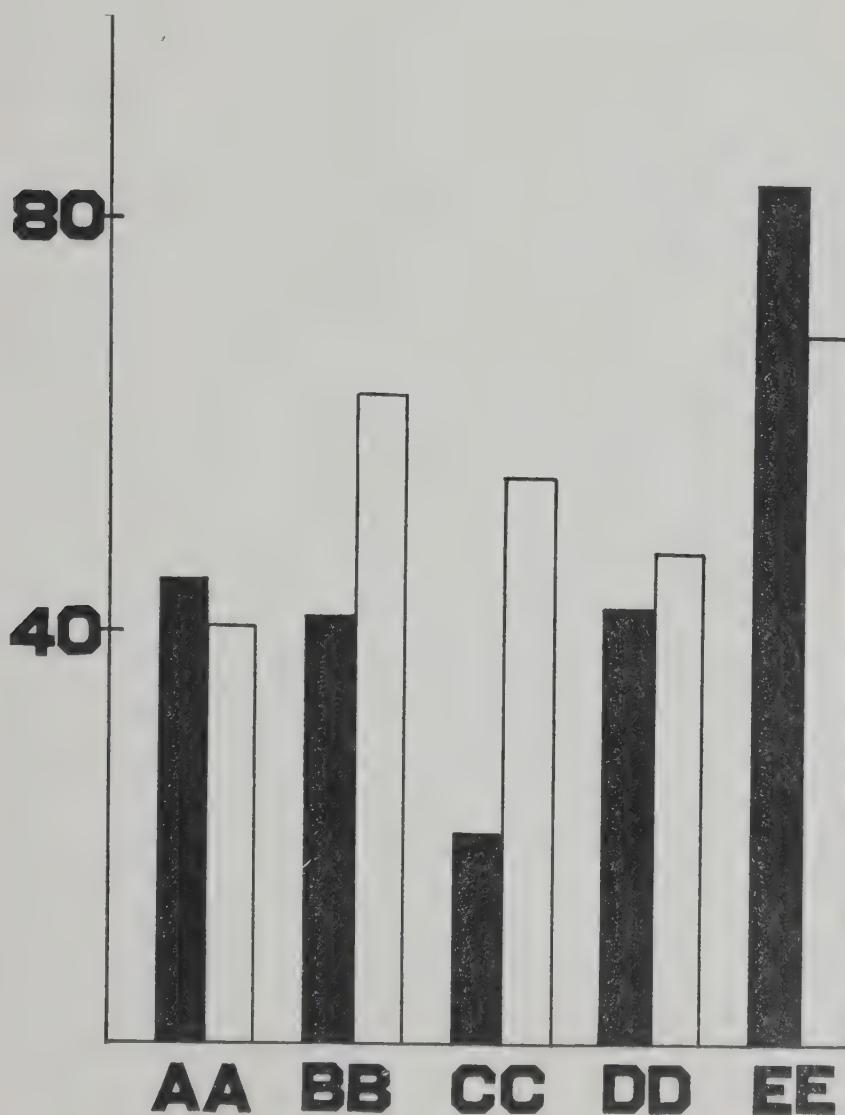


Diagram 1. Representation of the comparative growth of walnut seedlings from cultures in which different sodium salts were present in equivalent amounts. Shaded columns represent fresh weight of tops; unshaded columns, fresh weight of roots. AA, sodium nitrate; BB, sodium chlorid; CC, sodium bicarbonate; DD, sodium sulfate; EE, controls. Vertical scale represents green weight in grams.

The growth of the epicotyls may indicate the comparative toxicity of the various solutions, since practically no epicotyls had developed when the seedlings were placed in the cultures. Their development was most retarded in the  $\text{NaHCO}_3$  series. In the  $\text{NaNO}_3$  and the  $\text{NaCl}$  series, the margins of some of the leaves died showing what may be called salt burn. In the  $\text{NaHCO}_3$  series the leaves on the epicotyls were small, and in many cases the epicotyls did not emerge from the seed.

The primary roots (initially 8 to 15 cm. long) made small growth in all the Na series. The laterals produced later were short and swollen at the tips like those shown in other experiments with toxic salts. When the experiment was ended the roots in the toxic solutions were brown, though still living. The plants in the control series had well developed leaves and healthy white roots. Some idea of the toxicity of 1000 p.p.m. Na may be obtained by comparing the growth of seedlings in the  $\text{NaNO}_3$  series with that in the control series. In the former there was a  $\text{NO}_3$  concentration of 2692 p.p.m., while in the latter the  $\text{NO}_3$  concentration was 2872 p.p.m. In another experiment a concentration of 2692 p.p.m.  $\text{NO}_3$  added to the same nutrient solution in the form of  $\text{Ca}(\text{NO}_3)_2$  gave as good growth as the control solution.

The analyses recorded in table 6 show that the Na content of the tops and roots was high and rather uniform when the seedlings had grown in the  $\text{NaNO}_3$ ,  $\text{NaCl}$ , or  $\text{Na}_2\text{SO}_4$  series. Those grown in  $\text{NaHCO}_3$  had more Na in their tops than the other plants, but had practically the same percentage in the roots. It will be noted that the tops which made the least growth had the most Na in their ash. The ash of plants from the control series contained approximately the same percentage of Na as one usually finds in similar plants which have grown in less concentrated nutrient solutions.

The K content of the tops was reasonably uniform, though there was some reduction in the K content of the roots.

The effects of  $\text{NaNO}_3$ ,  $\text{NaCl}$ , and  $\text{Na}_2\text{SO}_4$  were not widely different, but  $\text{NaHCO}_3$  was more toxic than the other three salts used. Both the tops and roots of the seedlings showed qualitative differences which were not always evident from the weights of those parts. We have found that seasonal conditions may influence the results.

An earlier experiment in which nontoxic concentrations of salts were employed may be cited.<sup>20</sup> Rough-lemon seedlings were grown in nutrient solution containing equivalent concentrations of calcium



in conjunction with  $\text{NO}_3$ ,  $\text{SO}_4$ ,  $\text{Cl}$ , and  $\text{CO}_3$ . In the concentrations employed, the growth of roots was influenced more by the amount of calcium in solution than by the character of the anion with which the calcium was combined.

The effects of anions upon the growth of oat seedlings reported by Plate<sup>15</sup> afford an interesting comparison, although he used dilute acids rather than salts, and root growth was inhibited in all the solutions. The size of the oat shoot at the end of the fourteenth day in the different solutions was as follows:  $\text{PO}_4 > \text{SO}_4 > \text{NO}_3 > \text{Cl}$ .

## VI. THE EFFECT OF ALKALINITY ON GROWTH AND SAP REACTION

### *a. Walnut seedlings.*

The object of this experiment was to test the effect of a  $\text{Ca}(\text{OH})_2$  solution maintained at  $P_H$  9.0 upon the reaction of the sap of roots and tops. Two jars of 40-liter capacity were filled with tap water to which  $\text{Ca}(\text{OH})_2$  solution was added. Twelve walnut seedlings were placed in each jar through perforated covers so that their roots were immersed in the solution. They grew from April 13 to May 9. The  $P_H$  of the solution was maintained at or very near 9.0 by allowing a slow stream of saturated  $\text{Ca}(\text{OH})_2$  solution to flow continually into the culture solution.<sup>21</sup> The  $P_H$  of the culture solution was tested at least three times a day and the flow of the  $\text{Ca}(\text{OH})_2$  solution was regulated accordingly.

During the 26 days of this experiment the plants showed no injury, the roots were well developed though the tops seemed spindling. The fresh weights of tops and roots of 10 seedlings were 53 and 92 grams, respectively. The  $P_H$  of the juice expressed from the tops and roots was determined by means of the hydrogen electrode, and was 5.26 and 5.48, respectively. The acid nature of the plant juices agrees with our former determinations and with those of Theron.<sup>25</sup> It seems that the plant contains substances capable of acting as buffers which prevent the change of reaction in the sap.

Although the per cents of Ca found in ash of the tops and roots were 14.64 and 9.80, respectively, nevertheless the  $P_H$  of their sap showed no significant changes from the values obtained when such plants are grown in complete culture solutions.

In another experiment walnut seedlings were grown in tap water containing enough  $\text{Ca}(\text{OH})_2$  to raise the  $P_H$  considerably above 9.0. Within a few hours the roots were badly discolored; shortly afterwards



the laterals and the apical portion of the main root died. No new additions of  $\text{Ca}(\text{OH})_2$  solution were made and four days later the  $P_H$  of the solution had fallen to 7.2.

After the extreme alkaline conditions disappeared new laterals were produced not only from the white portions of the primary roots of the seedlings but also from the discolored parts of the primary roots, indicating that in the injured portion at least parts of the vascular system were still alive. The subsequent growth of laterals was very good. The original injury appears to have been confined to the cells of the parenchyma and meristem. Supplementary evidence on this question was afforded by the recovery of roots which had been held for a short time in culture solutions having a slightly injurious reaction. The surface of the roots was discolored. In a short time the reaction was brought to a  $P_H$  near 9.0 at which the roots resumed growth.

The subsequent elongation of the central column of root cells broke the discolored layer of cortical cells in the region of greatest growth and produced a banded surface. Similar injury and recovery has been observed when roots had been kept in solutions containing chlorids or other deleterious salts.

#### *b. Wheat seedlings.*

Four 40-liter jars were planted with small wheat seedlings. Two culture jars contained a modified nutrient solution maintained as near  $P_H$  6 as possible, the other two jars contained the same modified solution to which enough  $\text{Ca}(\text{OH})_2$  solution was added to maintain a  $P_H$  of 8.0. The composition of the modified nutrient solution expressed as p.p.m. was as follows:

Na	K	Ca	Mg	Fe	Cl	$\text{NO}_3$	$\text{SO}_4$	$\text{PO}_4$	Total
7	496	33	54	1	10	819	216	105	1741

The  $P_H$  of the solutions was adjusted three times each day and the solutions were renewed every two or three weeks during the two months of the experiment. Sufficient ferric tartrate was added periodically to prevent the appearance of chlorosis.

After two months the green weight of 20 plants from the culture at  $P_H$  6.0 was 209 g.; the same number from the culture at  $P_H$  8.0 weighed 498 g. It is quite likely that part of the increased growth at the higher  $P_H$  was due to increased supply of Ca. The plants from both cultures appeared healthy.

The data in table 7 show that there was no effect on the  $P_H$  of tops due to the addition of  $Ca(OH)_2$  to the culture solution, but that the  $P_H$  of roots was slightly affected. Hurd<sup>7</sup> and Haas<sup>2</sup> have reported an increased  $P_H$  in the tops of wheat plants grown in soil to which calcium carbonate was added. On the other hand Newton<sup>14</sup> and the writers found no such increase when plants were grown in water cultures. It would appear that the plants contain substances acting as buffers which maintain a constancy of reaction in spite of differences in the reaction of the medium or of changes in the ion composition of the sap. Similar conclusions were given in another paragraph based upon studies of the sap of walnut seedlings grown at different  $P_H$ .

TABLE 7  
THE EFFECT OF ADDED  $Ca(OH)_2$  UPON WHEAT SEEDLINGS

$P_H$ of culture solution.....	6.0	8.0
Green weight of tops per 20 plants.....	173 g.	440 g.
Green weight of roots per 20 plants.....	36 g.	58 g.
$P_H$ of sap of tops.....	6.1	6.1
$P_H$ of sap of roots.....	6.7	7.2
Calcium content of 20 c.c. sap from tops.....	.0017 g.	.0027 g.

VII. THE RELATION OF H-ION CONCENTRATION TO THE ABSORPTION OF CHLORIDS

a. *Citrus seedlings.*

The experiments were designed to determine the amount of chlorin absorbed by citrus seedlings from solutions of various H-ion concentration. Rough-lemon seedlings (*Citrus limonia* Osbeck) were grown and all of the plants were from seeds of a single tree. The cultures were grown in enameled-ware pails of ten liters capacity, each of which had approximately 65 seedlings supported on a perforated wooden cover (fig. 8). The solutions were renewed at intervals of three to four days and their reactions were adjusted three times each day.\*

The culture solutions was based on that employed by Hoagland. Sodium chlorid was added in amounts equivalent to 1000 p.p.m. (table

\* The writers are indebted to A. E. Michelbacher for the careful manipulation of details connected with this work.



8), and hydrates were added to produce the desired concentration of OH-ions. The concentration of sodium chlorid employed is not detrimental to the growth of citrus seedlings in the time involved in these experiments.

TABLE 8

COMPOSITION OF NUTRIENT SOLUTION TO WHICH 1000 p.p.m. NaCl HAD BEEN ADDED

Parts per million										
Na	K	Ca	Mg	Fe	Mn	Cl	NO <sub>3</sub>	SO <sub>4</sub>	PO <sub>4</sub>	Total concentration
400	185	159	54	1	0.1	617	718	216	105	2455.1

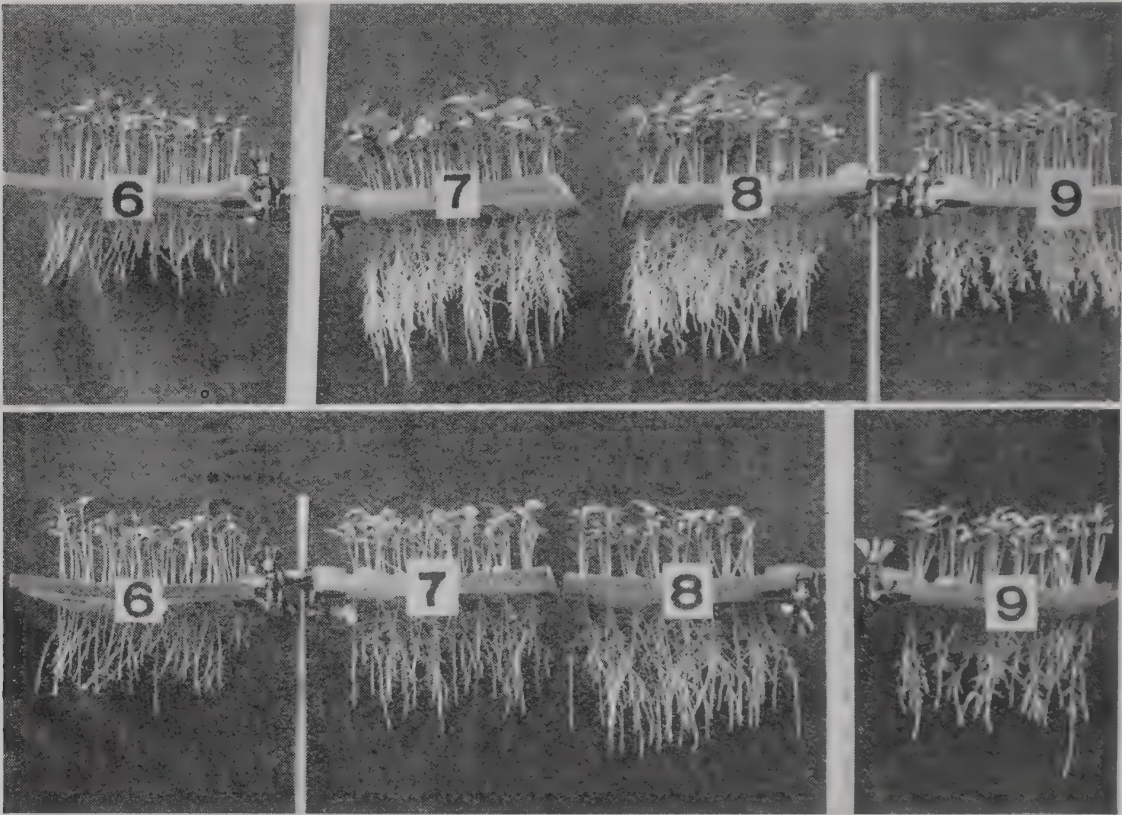


Fig. 8. Rough-lemon seedlings grown in water cultures maintained at different hydrogen-ion concentrations. Numbers indicate  $P_H$  values maintained. Plants in upper row were taken from cultures in which calcium hydrate was used to maintain  $P_H$  values above 6.0; in lower row sodium hydrate was used.

In the first experiment (series FF, table 9), begun on March 14, rough-lemon seedlings were placed in four solutions of  $P_H$  6, 7, 8, and 9. The  $P_H$  at which it was possible to maintain the original solution for a few hours with no additions of acid or alkali ranged between 5.5 and 6.0 but usually was close to  $P_H$  6, and we have here designated this solution as  $P_H$  6. The three higher  $P_H$  values were maintained by the frequent addition of calcium hydrate. The second experiment (series GG, table 9) was begun March 22, using seedlings from the



TABLE 9  
AMOUNTS OF VARIOUS IONS PER 100 ROUGH-LEMON SEEDLINGS GROWN IN HOAGLAND'S SOLUTION  
+1000 p.p.m. NaCl AT VARIOUS P<sub>H</sub> VALUES

Series	FF				GG				Amounts of various ions in 100 rough-lemon seedlings, cotyledons included, taken from germinating box of sphagnum moss.
	Ca(OH) <sub>2</sub> used to raise P <sub>H</sub> value above 6.0 Seedlings 31 days old				NaOH used to raise P <sub>H</sub> value above 6.0 Seedlings 23 days old				
	6.0	7.0	8.0	9.0	6.0	7.0	8.0	9.0	
P <sub>H</sub> of culture solutions.....									
Green weight.....	g. 33.30	g. 61.40	g. 72.30	g. 46.90	g. 28.80	g. 29.40	g. 39.40	g. 31.90	g. 21.80
Dry weight.....	7.02	9.44	10.67	8.12	5.40	3.99	5.25	5.23	2.53
Ash.....	.648	1.420	1.367	.952	.502	.532	.680	.580	.197
Na.....	.072	.135	.147	.090	.042	.058	.062	.065	.020
K.....	.145	.258	.306	.212	.118	.121	.168	.141	.055
Ca.....	.041	.165	.105	.090	.032	.045	.048	.032	.011
Mg.....	.015	.028	.027	.020	.012	.013	.014	.014	.006
Cl.....	.051	.115	.132	.096	.036	.037	.054	.056	.004
SO <sub>4</sub> .....	.037	.094	.098	.042	.027	.039	.047	.036	.014
PO <sub>4</sub> .....	.095	.211	.164	.075	.084	.089	.068	.056	.054

same lot as those used in the first. Here the three higher  $P_H$  values were obtained by the addition of sodium hydrate. On April 14 the seedlings were photographed, harvested and prepared for analysis.

Figure 8 shows the growth in the two series. The former seedlings were grown 31 days and were therefore larger than the latter which were grown 23 days. The seedlings grown in culture solutions maintained at  $P_H$  7, 8, and 9 showed marked improvement over those grown at  $P_H$  6.

The seedlings grown at  $P_H$  6 although they grew slowly, made practically the same type of growth as that simultaneously obtained with unmodified Hoagland's solution. The seedlings put out one or two pairs of new leaves which had a good color and were vigorous in every respect. The greatest growth of tops was obtained at  $P_H$  8 in both sets. The tops of seedlings at  $P_H$  8 were superior to those at  $P_H$  6, but the differences between those at  $P_H$  6, 7, and 9 are not significant. The greatest contrast was in the growth of the root systems. In the  $\text{Ca}(\text{OH})_2$  series the roots developed richly branching systems at  $P_H$  7 and 8, but at  $P_H$  9 the growth was more limited. In the NaOH series the roots were superior at the higher  $P_H$  values to those in the culture at  $P_H$  6.

Table 9 shows the response of the rough-lemon seedlings to the several culture solutions used. The data represent the weight of seedlings and their composition, in grams per 100 seedlings. The initial composition of seedlings is shown in the last column in the table and serves as a means of judging the amount of absorption which occurred.

The weights and photographs show that the plants made good growth in all cases, yet the higher hydroxyl-ion concentration appears to have stimulated growth. In series FF part of the increased growth at  $P_H$  8.0 might have been due to the increased amount of calcium present, but this explanation does not apply to series GG.

The increased growth of seedlings in solutions above  $P_H$  6.0 was generally accompanied by an increase in the amounts of various ions absorbed. It appears that plants grown at  $P_H$  7.0 and 8.0 absorbed slightly greater amounts of sodium than those grown at  $P_H$  6 in both series, while those at  $P_H$  9.0 showed a very small increase. In series FF the plants at  $P_H$  7.0 absorbed twice as much chlorin as those grown at  $P_H$  6.0. At  $P_H$  8.0 there was a still greater absorption, but at  $P_H$  9.0 it was less than at  $P_H$  7.0. In series GG similar amounts of chlorin were absorbed at  $P_H$  6.0 and 7.0, with an increase at  $P_H$  8.0 and 9.0.

The net absorption of sodium and chlorin for the 31-day period is represented by graphs in diagram 2, together with the ash and the green weight per 100 plants. The data show that these seedlings absorbed a larger quantity of both anions and cations from neutral or alkaline solutions than from the slightly acid solution, and made a correspondingly greater growth.

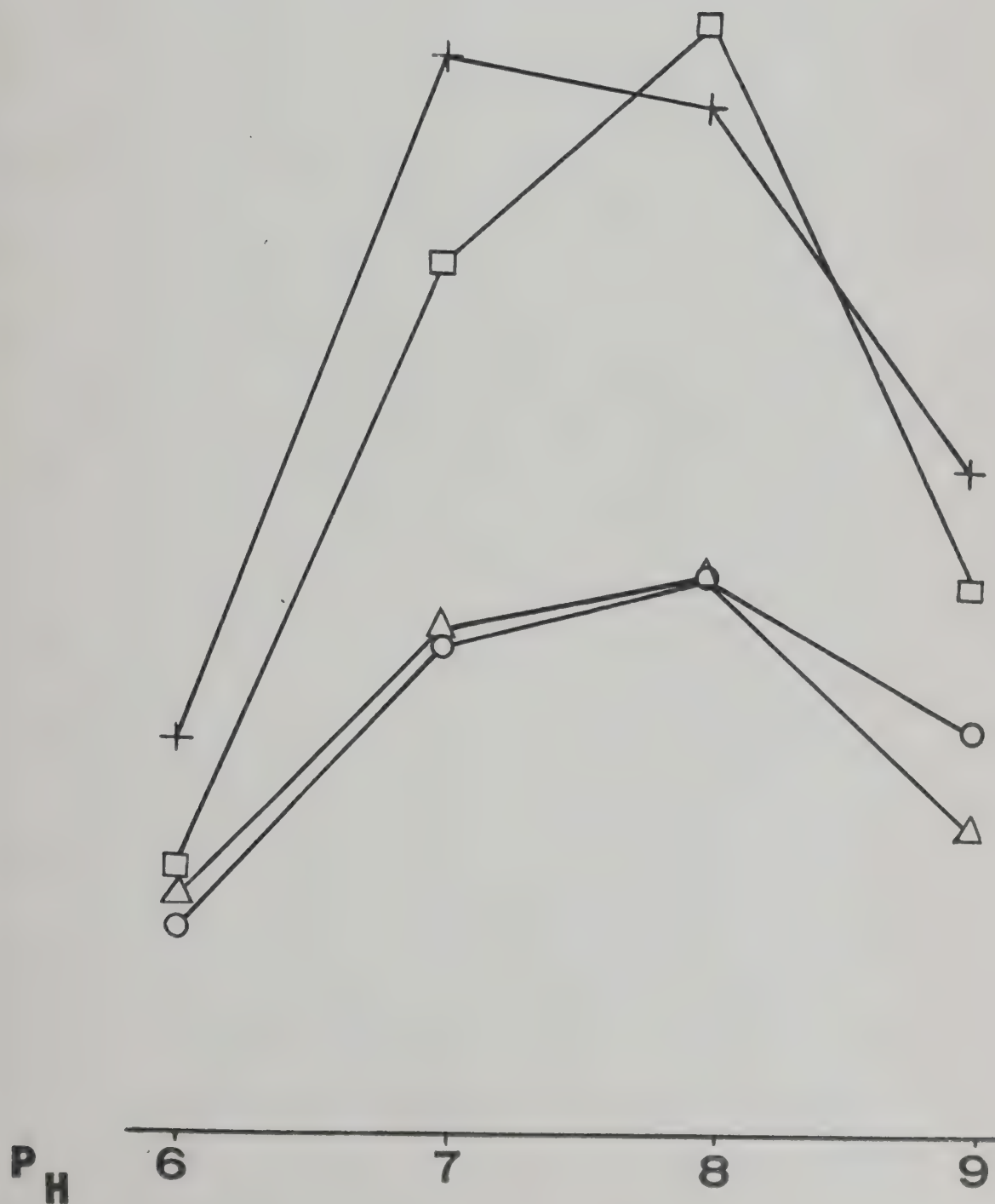


Diagram 2. Growth and absorption of rough-lemon seedlings in nutrient solutions of different  $P_H$  values.  $\square$ , green weight of plants  $\div 200$ ;  $+$ , weight of ash  $\div 5$ ;  $\Delta$ , sodium;  $O$ , chlorin.



Table 10 shows the composition of the tops and roots of the same seedlings, expressed as percentages of the ash. The percentage of ash in the tops and roots of both series was greatest when grown in solutions with  $P_H$  values of 7.0 and 8.0. It should be noted that in this case, and in some others the percentages were greater at  $P_H$  9.0 than at  $P_H$  6.0.

The percentage of cations in the ash (table 10) shows little evidence for the idea that plants absorb relatively more cations from alkaline solutions. The tops and roots of series FF showed no significant differences in the percentage of sodium at different  $P_H$  values. In series GG the plants from the solutions of higher  $P_H$  values were in five cases out of six somewhat richer in sodium. It will be remembered, however, that the  $P_H$  of these solutions was raised by the addition of NaOH, and that their sodium content was somewhat increased.

With the possible exception of the tops of series GG kept at  $P_H$  8, the percentage of calcium showed a marked increase when the plants were grown in solutions of  $P_H$  7.0. This fact is more noteworthy when we remember that the higher  $P_H$  values in series FF were produced by the addition of  $\text{Ca}(\text{OH})_2$ .

The percentage of chlorin in the ash of the tops is smaller in the plants grown in the solution having an acid reaction than in those grown in neutral or alkaline solutions. The smallest percentage of chlorin in both series was found in the roots which grew in the neutral solution, while the percentages in other solutions were quite uniform.

With the exception of the roots in series FF the percentage of sulfate was greatest in plants grown at  $P_H$  7.0 or 8.0. In series GG the percentages of sulfate in the ash of tops and roots were approximately the same at  $P_H$  6.0 and  $P_H$  9.0.

The results presented in this paper open a field in which profitable work may be done with other species of plants and in other ranges of hydrogen and hydroxyl-ion concentrations. It is more than probable that acid or alkaline conditions affect, not only the equilibria between the components of the soil, but the equilibrium between the soil solution and the plant.

Some of Theron's results<sup>25</sup> indicate that the amount of growth and the kind of plants used as well as the ratio of certain ions in the solution may be factors affecting the absorption of the various ions at the different  $P_H$  values. The evidence previously published indicates that optimum culture solutions for plants generally have a  $P_H$  value of less

TABLE 10

COMPOSITION OF ROUGH-LEMON SEEDLINGS GROWN IN HOAGLAND'S SOLUTION + 1000 p.p.m. NaCl AT VARIOUS P<sub>H</sub> VALUES

Series		FF				GG			
		Ca(OH) <sub>2</sub> used to raise P <sub>H</sub> above 6.0 Seedlings 31 days old				NaOH used to raise P <sub>H</sub> above 6.0 Seedlings 23 days old			
P <sub>H</sub> of culture solutions.....		6.0	7.0	8.0	9.0	6.0	7.0	8.0	9.0
Tops.....	Per cent ash in dry matter.....	9.08	12.56	12.16	11.30	9.06	12.90	12.83	11.37
	Na.....	12.37	12.73	11.51	11.09	8.85	10.06	8.00	11.78
	K.....	18.74	17.78	17.99	17.50	19.96	19.89	19.72	18.36
	Ca.....	7.37	11.41	9.16	9.09	7.65	8.68	9.14	6.26
	Mg.....	2.34	2.30	1.93	1.72	2.31	2.37	2.25	2.14
	Cl.....	6.52	8.99	7.88	8.99	5.14	5.55	5.31	8.07
	SO <sub>4</sub> .....	2.68	4.45	3.94	2.52	2.90	3.93	3.16	3.05
	PO <sub>4</sub> .....	12.31	10.93	10.13	8.28	14.78	13.51	11.01	9.49
Roots.....	Per cent ash in dry matter.....	9.69	20.90	14.22	12.44	9.93	14.49	13.18	10.91
	Na.....	7.90	4.11	9.53	6.48	7.47	13.02	11.52	9.46
	K.....	32.77	19.27	32.00	31.15	31.93	28.97	35.35	35.59
	Ca.....	3.33	12.55	4.92	9.93	3.58	7.63	2.92	3.74
	Mg.....	2.13	1.60	2.02	2.53	2.40	2.12	2.00	2.76
	Cl.....	12.37	7.48	12.20	12.24	12.11	9.90	13.63	12.42
	SO <sub>4</sub> .....	14.07	9.74	13.52	8.00	11.59	14.90	14.90	12.42
	PO <sub>4</sub> .....	21.22	20.75	15.67	7.11	21.40	23.54	8.10	9.23



than 7.0, and that OH ions are more harmful than equivalent concentrations of H ions. It is worth noting that the previous experimental work was largely done with seedlings of cereals or legumes, and it is not surprising to find that plants of another genus may react differently. Within a certain range of  $P_H$  values a given concentration of OH ions was less harmful to rough-lemon seedlings than an equivalent concentration of H ions. The graphs in diagram 2 show a certain consistency between green weight, ash, sodium, and chlorin content at different  $P_H$  values. These data, together with the appearance of the plants, indicate that conditions for absorption and growth were definitely superior in the solution of  $P_H$  8.0.

*b. Walnut seedlings.*

Walnut seedling cultures were made in 40-liter jars containing Hoagland's solution to which 1000 p.p.m. NaCl was added. The higher  $P_H$  values in one set were maintained by the addition of NaOH, and in the other by  $\text{Ca}(\text{OH})_2$ . The solutions were changed at intervals of 10 to 14 days and the reactions were adjusted thrice daily. Twelve seedlings were put into each solution on March 27 and allowed to grow until May 9. The plants in all cultures made uniformly good growth as shown by the fresh weights of plants (table 11).

The ash of tops and roots of plants from the different solutions had a rather uniform Cl content, although the roots were uniformly richer in Cl than the tops. It would appear from these figures that the H-ion concentration of the media had no appreciable effect either upon the growth of the plants or upon their chlorin absorption.

The sap was pressed from the tops and roots of three plants from each culture. The  $P_H$ , as determined by the use of the hydrogen electrode (table 11), shows no significant differences. Hoagland and Davis<sup>6</sup> found a similar constancy of  $P_H$  in the sap of *Nitella* from solutions whose  $P_H$  ranged from 5.0 to 9.0.

The analysis of the ash of the walnut seedlings also showed remarkable constancy of composition in most cases. Hoagland<sup>5</sup> reports similarly that there was no general relation between the reaction of the plant sap and the nature of the ion which the plant had absorbed in excess. There is no evidence in these figures for the earlier, and frequently repeated, idea that Na and K are to a certain extent interchangeable in the plant. The percentages of K and Mg showed no significant variations corresponding to the  $P_H$  of the culture solutions.



The idea that proteins may combine with anions or cations, according to the hydrogen-ion concentration of the solution, has led some workers to assume that acid solutions bathing plant roots favor anion absorption and that alkaline solutions favor cation absorption. Our

TABLE 11

EFFECT OF P<sub>H</sub> OF CULTURE SOLUTION UPON GROWTH AND ABSORPTION BY WALNUT SEEDLINGS

P <sub>H</sub> of culture solution	Series HH							
	6.0 to 6.5		7.0		8.0		9.0	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Fresh weight per 10 plants (grams).....	93	110						
NaOH series.....			80	95	95	123	81	107
Ca(OH) <sub>2</sub> series.....			78	121	93	102	70	106
P <sub>H</sub> of the sap of the plants.....	5.31	5.68						
NaOH series.....			5.48	5.83	5.31	5.82	5.31	5.87
Ca(OH) <sub>2</sub> series.....			5.36	5.75	5.43	5.75	5.26	5.77
Per cent Na in the ash....	8.72	17.03						
NaOH series.....			9.93	20.81	10.64	21.30	10.45	19.96
Ca(OH) <sub>2</sub> series.....			10.22	15.21	8.53	17.13	9.65	16.77
Per cent K in the ash.....	27.77	21.87						
NaOH series.....			30.34	19.82	30.61	20.16	33.07	18.77
Ca(OH) <sub>2</sub> series.....			29.80	21.79	29.59	23.81	31.60	21.01
Per cent Ca in the ash....	11.18	7.36						
NaOH series.....			9.76	7.87	9.23	6.42	7.08	7.01
Ca(OH) <sub>2</sub> series.....			10.90	9.05	12.00	7.44	10.27	8.48
Per cent Mg in the ash...	5.24	2.44						
NaOH series.....			5.05	1.62	5.03	2.56	5.01	2.67
Ca(OH) <sub>2</sub> series.....			5.15	2.55	4.90	2.49	4.81	2.60
Per cent Cl in ash of plants.....	4.98	8.10						
NaOH series.....			4.87	7.45	4.92	7.78	4.96	8.97
Ca(OH) <sub>2</sub> series.....			4.04	7.20	3.36	8.15	4.00	8.71

results, however, seem to indicate that the neutral point (P<sub>H</sub> 7.0) is not a point of great importance so far as absorption by plants is concerned. In other words, there is no evident reason for assuming that the neutral point of distilled water is a sort of turning point, or isoelectric point, in regard to absorption of ions from a solution by plant roots.

# VIII. THE EFFECTS OF SODIUM CHLORID ON ORANGE TREES IN SOIL, AND THE RESULTS OF LEACHING

When citrus trees are grown in soils containing harmful amounts of saline material, the leaves are often the first organs to show injury. For a time the leaves appear yellowish, then the margins and apical regions die, and finally an excessive abscission of leaves occurs which makes the unhealthy condition of the trees very conspicuous. Loughridge<sup>12</sup> and Hilgard<sup>3</sup> were among the first to emphasize the toxicity of

TABLE 12

VOLUME OF LIQUIDS APPLIED TO THE CULTURES, AND TRANSPIRATION OF  
ORANGE TREES

(Figures in parenthesis were not included in making averages)

Tree	Volume of liquids added (liters)					Drainage water (liters)	Trans- piration (liters)	Ratio of transpira- tion to dry weight of tree
	Nutrient solution	NaCl solution	Distilled water	Tap water	Total			
84	60	.....	81	274	415	0	415	436
85	(30) <sup>o</sup>	.....	(77)	(146)	(253)	0	(253)	(357)
86	63	.....	93	304	460	0	460	465
87	60	.....	103	328	491	0	491	.....
88	60	.....	104	306	470	0	470	381
89	69	40	33	58	200	46	154	.....
90	69	42	39	173	323	31	292	603
91	66	39	45	212	362	31	331	519
92	66	24	37	204	331	31	300	472

sodium chlorid to citrus trees. More recently Kelley and Thomas<sup>9</sup> have added to our knowledge of the effects of saline irrigation water. The present writers<sup>18</sup> have described the results produced experimentally when young orange trees were grown in sand cultures to which solutions of sodium chlorid were regularly added. We have noted that the chlorophyll of the leaves had a tendency to fade, and that premature abscission was the rule, except where sufficient amounts of calcium salts were present. Twigs and roots were restricted in their growth and eventually many were killed. Rudolfs<sup>24</sup> has reported similar effects of sodium chlorid on other species of trees.

In the following pages we give additional data on the effects experimentally produced by the addition of sodium chlorid to sand and to soil cultures, with especial reference to the chlorid content of orange



leaves, and to the changes which occurred when the soil impregnated with salt was leached with water. The importance of the results lies largely in the fact that we have here produced definite symptoms by the application of a known cause.

#### *A. Trees under controlled conditions.*

Young orange (*Citrus sinensis*) trees were grown in large galvanized-iron cans containing sand or soil under conditions which permitted of simple and convenient observation. The details of the general cultural methods have been given elsewhere.<sup>17</sup> The trees were planted May 20, 1920, and removed in February, 1922.

##### *1. Sodium chlorid added to non-saline soil.*

Trees 13 and 16 were grown in silica sand which received nutrient solution plus 1000' parts per million of sodium chlorid. The other trees were grown in cans which contained soil taken from an uncultivated area on the Citrus Experiment Station property. The soil has been classified as Sierra loam and occurs in regions devoted to citrus culture in the vicinity of Riverside.

Trees 84-88 grew in the soil described above and were irrigated for the first eleven months with distilled water. Trees 89-92 were grown in soil from the same source and were irrigated for the same period with a solution containing 1500 parts per million of sodium chlorid. The latter were seriously injured and showed symptoms which will be described later. During the first eleven months there was no drainage water from the soil. In the latter part of April, 1921, cultures 89-92 were leached with tap water until, as shown in table 12, 30 to 46 liters had been recovered from each container. From that time nutrient solution was added to all cultures to furnish the trees a more nearly optimum supply of nutrient ions.

##### *(a) Changes in the soil.*

When the soil from cultures irrigated for five months with distilled water was sampled on October 8, the amount of material extracted with distilled water (table 13) was about half that obtained five months earlier. The concentration of  $\text{HCO}_3$  ions was much diminished and the  $\text{NO}_3$  concentration increased from nothing to an appreciable amount. The concentrations of  $\text{SO}_4$  and of Cl anions showed no significant change.



The solutes extractable with water eleven months after installing the cultures showed slight differences in concentration from those observed at the expiration of five months. The soil of cans 89-92 gave extracts which contained more Ca, Mg, Na, and Cl than that from cultures receiving distilled water. At the end of eleven months their total content of soluble solids had increased from 589 to 1033 parts per million but the  $P_H$  of the extract showed little change.

TABLE 13

ANALYSIS OF SOIL USED IN EXPERIMENTS ON THE EFFECT OF SODIUM CHLORID  
(Calculated on the basis of air-dry soil)

	Samples taken when experiment was installed	Cultures 84-88, which received distilled water		Cultures 89-92, which received 1500 p.p.m. sodium chlorid	
	May 20, 1920	Oct. 8, 1920	Apr. 14, 1921	Oct. 8, 1920	Apr. 14, 1921
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
SiO <sub>2</sub> .....	.....	27	26	25	42
Ca.....	.....	28	24	53	91
Mg.....	.....	9	9	16	28
Na.....	.....	13	22	107	191
HCO <sub>3</sub> .....	183	64	101	61	115
CO <sub>3</sub> .....	0				
Cl.....	18	24	28	221	436
NO <sub>3</sub> .....	0	66	11	58	48
SO <sub>4</sub> .....	22	19	10	15	33
PO <sub>4</sub> .....	18				
Total solids as sulphates...	400	178	194	589	1033
$P_H$ .....	.....	6.7	6.8	6.7	7.0

The soil in cultures 89-92 was leached with distilled water on April 15, 1921, until percolates began to appear. Only the first portion of the percolate was alkaline to phenolphthalein, although subsequent portions were alkaline to other indicators. A filtered sample of the first 500 c.c. of percolate from culture 92 showed 12 parts per million CO<sub>3</sub> and 67 parts per million of HCO<sub>3</sub>. On the following day the percolates obtained from cans 89 and 92 were still alkaline to phenolphthalein. The application of additional quantities of distilled water followed by nutrient solution finally gave percolates which had  $P_H$  values below 8.3.

The first portions of the percolates from cans 87 and 88 showed no alkalinity to phenolphthalein. The results of this and subsequent determinations are shown in table 14. The third column of table 14 contains the  $P_H$  values of the percolates obtained at the conclusion of the leaching process which had been in progress for three weeks.

The concentration of chlorin in the percolate varied considerably from time to time. The chlorin content of the percolate from can 92 was small, more or less in keeping with the relative amount of sodium chlorid added. The point of interest was the relatively extensive and the long continued formation of black alkali in the soil of this container in comparison with the soils of cans 90 and 91, which received considerably larger amounts of the salt.

TABLE 14  
DATA ON THE PERCOLATES FROM VARIOUS CULTURES

Culture	Grams of NaCl added	$P_H$ of percolates after continued leaching 21 days	Parts per million of chlorid in percolates		
			After 1 day	After 6 days	After 15 days
87	0.0	.....	25	.....	.....
88	0.0	.....	21	.....	.....
89	59.3	7.8	780	984	1179
90	63.4	6.8	255	1167	701
91	58.8	7.0	280	1186	423
92	35.5	7.8	248	44	199

Nutrient solution was added from time to time during the leaching process as well as afterward, and both distilled and tap water were used to supply the soil moisture after the salt treatment was discontinued.

(b) *Effects on the trees.*

The trees in cultures 84-88, which received only distilled water, grew fairly well for some time, but eventually showed the symptoms commonly observed when the supply of nitrates is insufficient. After a lapse of several months, the soils were irrigated from time to time with Hoagland's nutrient solution. The effect of the nutrient solution was shown in the improved appearance of the trees and in their increased growth. When the trees were removed from the cultures their condition was good and the weight of the various parts (table 15) exceeded that of trees grown simultaneously in sand receiving continuous applications of nutrient solution.

The trees in cultures 89-92 grew only a short time before they began to show the harmful effects of the sodium chlorid in the irrigation water. The leaves eventually became yellow and showed dead margins and tips which are characteristic of salt injury (fig. 9). These leaves fell after a time and were followed by a new crop which usually showed more actute symptoms of injury. The symptoms known as "mottle-leaf" did not appear on any of the trees receiving saline irrigation water. We may note in passing that the percolate from these

TABLE 15  
DATA ON THE TREES GROWN IN SOIL CULTURES  
(Figures in parenthesis not included in the averages)

Tree	Number of leaves on tree at conclusion of experiment	Dry weight (grams)					
		Leaves	Shoots	Trunk	Root	Rootlets	Total
84.....	995	224	132	171	197	227	951
85.....	(1042)	(206)	(96)	(141)	(135)	(130)	(708)
86.....	1172	311	126	234	184	234	989
87.....	(873)		(148)	(293)	(256)	(239)	
88.....	730	194	152	368	316	204	1234
Average.....	966	210	137	258	232	222	1058
89.....	(473)		(36)	(159)	(103)	(39)	
90.....	464	117	75	123	94	75	484
91.....	707	153	95	139	136	115	638
92.....	623	139	89	170	136	101	635
Average.....	598	136	86	144	122	97	586

cultures at the time when the injury was very severe contained 91 parts per million of calcium (table 13), which may have been responsible for the absence of mottle-leaf on these trees, although it was not sufficient to prevent tip burn. Cummins and Kelley<sup>1</sup> have reported that sodium salts, when applied to soil, set free calcium, and further applications of sodium salts brought about an excessive concentration of soluble sodium in the soil solution, and eventually the soil became deflocculated and impervious.

The depauperate leaves shown in fig. 9 are quite representative of the effects of saline irrigation water on these trees. None of them attained the size usually reached by leaves of the Valencia orange tree. The tissue on one side of the midrib frequently made less growth than



that on the other, with the resulting formation of asymmetrical leaves. In February, 1921 (two months prior to making the photograph), the leaves of trees 90 and 91 showed a considerable amount of tip burn and the older leaves were falling rapidly. Trees 89-92 produced a large number of flowers, in this respect resembling unhealthy trees in the field. The bark on the trunks of trees 89-92 was killed in areas near the surface of the soil. The dead areas were above the surface of the soil and could not have been due to contact with the saline

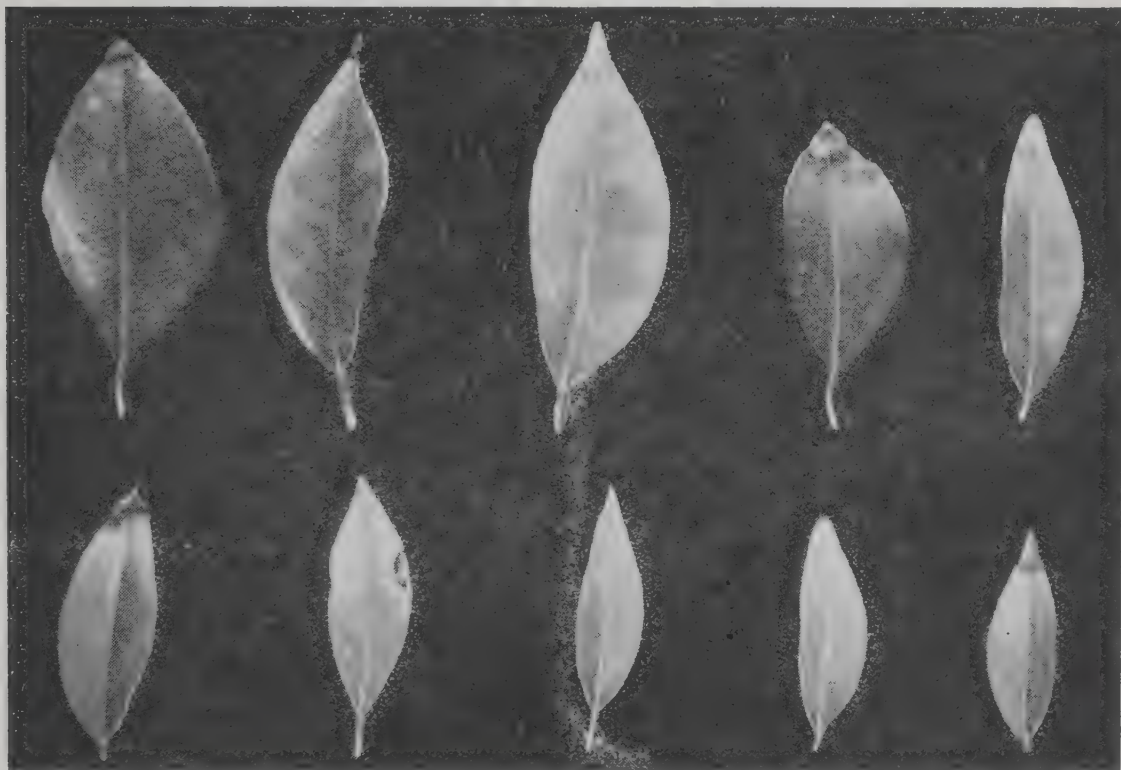


Fig. 9. Orange leaves from tree which was injured by applications of 1500 p.p.m. sodium chlorid.

irrigation water, since special care was used to prevent it from coming in contact with the trunks. The trunks of trees 84-88 were free from this type of injury. The shoot and root growth of trees 89-92 was notably limited.

In April, 1921, the soil of cans 89 to 92 was leached with distilled water. Leaching of the soil and removal of the drainage water were carried on while the trees were *in situ*, hence any ill effects due to saturation of the soil during the leaching process should have been evident. The leaching process greatly benefited the trees, as will be shown subsequently.

Shortly after the first drainage water was obtained, finely divided colloidal material appeared in it and the rate of percolation was

greatly reduced. Hoagland's nutrient solution was then added in small amounts from time to time, care being taken not to have an excess of solution standing for any long time on the surface of the soil. Frequent additions of solution and removals of drainage water were made, the method followed being in imitation of the removal of salts from precipitates on a filter.

The leaching process naturally removed salts which would have been useful to the trees, and the addition of nutrient solution was therefore advisable if the series were to be at all comparable with that which received no sodium chlorid. The total volume of nutrient solution applied to the soils in series 89-92 was somewhat in excess of that given to the other series (table 12), nevertheless it did not produce in the ten months during which no sodium chlorid was

TABLE 16  
P<sub>H</sub> AND OSMOTIC PRESSURE OF THE SAP OF MATURE LEAVES

Series	P <sub>H</sub>	Osmotic pressure (average of 2 determinations)
84-88.....°.....	5.80	21.03
89-92.....°.....	5.82	20.32

applied, trees equal to those produced in the other series. The dry weight of the trees was only about half that of the trees grown in the soil to which no sodium chlorid was added.

Determinations of the hydrogen-ion concentration and of the osmotic pressure of the sap of mature leaves were made May 26, 1921. The former was electrometrically determined and the latter was computed from the lowering of the freezing point. Table 16 shows no significant differences in the P<sub>H</sub> of the leaf sap, though as we have seen, the roots of the trees were growing in a soil which had received large concentrations of saline irrigation water and which upon being leached had shown black alkali in the drainage water. Rudolf's<sup>14</sup> has expressed the belief that chlorin increases the acidity in the plant cell, accelerating or harming the vital activities according to the amount employed, but the above results indicate that the chlorin had no appreciable effect on the P<sub>H</sub> of the plant sap. A large salt content in leaves may tend to keep a given P<sub>H</sub> more constant rather than to change the P<sub>H</sub> value.



The trees in both series made very satisfactory growth during the summer of 1921. The appearance of the trees on August 25 may be seen in fig. 11, which shows material improvement over the condition four months earlier (fig. 10). The new foliage produced in series 89-92 was free from the unfavorable characters shown previously.



Fig. 10. Trees in the experiment described. From left to right; trees 87, 88, 89, 90, and 91. Trees 87 and 88 grew eleven months in soil which received distilled water. The other trees grew in similar soil and received water containing 1500 parts per million of sodium chlorid.



Fig. 11. Trees in the experiment at the end of sixteen months. From left to right; trees 88, 89, 90, 91, and 92. The four trees on the right show recovery from the injury induced by the application of sodium chlorid to the soil, and resemble the typical control tree 88 (on the left).

The difference between the volume of water (or solution) added and that of the percolate has been reckoned as transpiration (table 12). While there may be some error in this procedure, it is common to all the trees. The amount of water lost by direct evaporation from the cans was small in comparison with that transpired by the foliage of



the trees. The ratio of transpiration to dry weight is somewhat greater in the case of the less thrifty trees in series 89-92. Data in table 16 show that the osmotic pressure of the sap of the leaves of the control series was not significantly greater than that of the sap of the injured trees at the time when leaching began.

(c) *The chlorin content of the leaves.*

Broadly speaking we may say that the addition of sodium chlorid to the soil produced no significant changes in the composition of the ash of the leaves except in respect to their sodium and chlorin content (table 17). Without further evidence we are not justified in assuming that there was a substitution of sodium for potassium.

TABLE 17

THE EFFECT OF SODIUM CHLORID UPON THE COMPOSITION OF ORANGE LEAVES  
(Expressed as a percentage of the ash)

	Sand cultures	Soil cultures		
	Trees 13-16	Trees 84-88	Trees 89-92	
	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Na in culture solution.....	400	0	590	590
Cl in culture solution.....	898	0	910	910
Condition of leaves.....	No injury.	No injury.	Injured.	Injured, about to fall.
Constituents of the ash:				
Na.....	1.61	1.64	4.38	3.98
K.....	38.25	8.82	9.86	8.85
Ca.....	8.77	29.77	28.55	26.85
Mg.....	1.15	2.36	2.78	2.65
Cl.....	4.73	0.28	17.03	21.36
SO <sub>4</sub> .....		2.36	2.27	2.55
PO <sub>4</sub> .....		3.71	5.26	3.88
Ash expressed as a per cent of dry matter.....	16.82	14.78	17.54	17.19

The ratio of soluble to insoluble constituents in the leaves was determined by extracting the dry-ground-leaf material with an excess of distilled water for several hours, filtering, and analyzing the soluble and insoluble portions separately. The ratio between the soluble and insoluble portions of each constituent is given in table 18. In the

case of the individual ions there is a significant difference in Na and PO<sub>4</sub> and a puzzling difference in the case of SO<sub>4</sub>. The others show no more variation than we are accustomed to find in this kind of work. The proportion of soluble PO<sub>4</sub> in the dried material bears a sort of inverse relationship to the total amounts present, although further evidence is necessary before we can attach much significance to these values.

TABLE 18  
THE RELATIVE SOLUBILITY OF THE ASH CONSTITUENTS OF VALENCIA ORANGE LEAVES  
GROWN IN SOIL. FIGURES REPRESENT PERCENTAGE OF THE TOTAL  
AMOUNT FOUND.

Source of leaves	Trees 84-88	Trees 89-92	
Condition of leaves	No injury	Injured	Injured, about to fall
	Soluble	Soluble	Soluble
Ash.....	67.51	70.80	71.15
Na.....	67.82	74.92	90.97
K.....	94.48	93.12	97.84
Ca.....	61.23	62.38	61.85
Mg.....	92.76	93.17	94.58
Cl.....	100.00	100.00	99.87
SO <sub>4</sub> .....	75.86	81.44	62.23
PO <sub>4</sub> .....	73.90	54.90	60.53

The percentage of soluble Cl is of great importance in view of the greatly increased amounts of this element which the injured leaves contained. The fact that all the Cl in the dried leaf material was soluble, gives some idea of the cause of the increasing deleterious effects which are associated with the absorption of chlorids. There is no evidence that the chlorin ions migrate from the leaves before abscission.

2. Soil previously made saline by orchard irrigation.

Valencia orange trees were also planted in cultures like those previously described to test the possibility of removing excessive amounts of salts under controlled conditions. The soil\* was obtained from an orange grove which had been injured by saline irrigation water of the following composition:

Ca	Mg	Na	Cl	CO <sub>3</sub>	HCO <sub>3</sub>	SO <sub>4</sub>	NO <sub>3</sub>	SiO <sub>2</sub>	Total solids as sulphates
172	58	228	627	0	213	76	28	39	1578

\* The soil on which the grove stands has been classified by the Bureau of Soils as Ramona loam.

When the soil samples were obtained from the grove we noted that practically all the functioning roots were found between 12 and 24 inches from the surface. Samples of the first 12 inches and of the second 12 inches were collected, each lot being kept separate. Sixteen large galvanized-iron cans were filled with the soil: five cans with the first foot layer, five with the second foot layer, and six with a mixture of equal parts of the two layers. A Valencia orange tree was planted in each can May 21, 1920. Three of the cans in each

TABLE 19  
COMPOSITION OF THE WATER EXTRACTS OF SALINE SOIL USED IN CULTURES FOUR MONTHS AFTER THE EXPERIMENT WAS BEGUN, EXPRESSED AS PARTS PER MILLION OF AIR-DRY SOIL

	First foot.		Second foot		Mixture of first and second foot	
	Unleached	Leached	Unleached	Leached	Unleached	Leached
Ca.....	325	95	15	8	132	50
Mg.....	100	34	5	2	42	20
Na.....	438	247	207	87	340	247
Cl.....	1002	390	200	89	566	334
CO <sub>3</sub> .....	0	0	0	0	0	0
HCO <sub>3</sub> .....	107	122	115	137	82	112
SO <sub>4</sub> .....	227	97	52	37	122	90
NO <sub>3</sub> .....	361	156	47	11	221	130
SiO <sub>2</sub> .....	46	48	56	45	45	45
Total solids as sulphates...	2950	1251	714	420	1710	1030
P <sub>H</sub> .....	7.0	7.0	7.0	6.8	6.9	7.0
Tap water added (liters)....	12.8	40.0	14.3	40.0	13.0	40.0
Drainage recovered (liters)	0	23.9	0	13.0	0	15.3

set were leached and the others were kept as near the optimum water content as possible. The amounts of soluble salt in the soil and the effect of leaching are shown by data in table 19. The total quantities of water and solution applied to the leached soils were about four times those given to the unleached soils. Analyses of the percolates showed a rather constant fall in chlorid content during the first year. Because of the effects of the leaching process upon the soil, we therefore applied a culture solution from time to time.

In a general way the growth of the trees in the several cultures reflected the content of soluble matter in the soils. The trees in the unleached first-foot soil were short lived. They sent out shoots which



TABLE 20  
VOLUME OF LIQUIDS ADDED AND GROWTH OF ORANGE TREES IN CULTURES IN SALINE SOIL

	Water applied	Nutrient solution applied	Average number of leaves on tree	Average dry weight per tree (grams)					
				Leaves	Shoots	Trunks	Roots	Rootlets	Total
	<i>Liters</i>	<i>Liters</i>							
First foot, leached <sup>1</sup> .....	268	57	704	123	77	145	127	110	582
Second foot, unleached.....	132	0	279	26	35	116	79	28	284
Second foot, leached.....	419	63	845	127	91	218	161	193	790
Mixture of first and second foot, unleached <sup>1</sup> .....	124	0	326	48	30	106	85	37	306
Mixture of first and second foot, leached <sup>2</sup> .....	360	60	754	143	96	128	123	195	685

<sup>1</sup> Two of the three trees in the set died and new trees were installed four months after the experiment was begun.  
<sup>2</sup> One of the three trees in the set failed to start growth and was replaced by a new tree four months after the experiment was begun.

reached a length of one or two inches, but failed to develop further. The leaves died before reaching their usual size; a little later the new shoots also died. No recovery occurred, in spite of careful attention. The trees in the other unleached soil made restricted growth, and showed marked evidence of salt injury quite similar to that observed where sodium chlorid was added. The leaves were small, yellowish, and dead at the tips. No tendency to mottle-leaf was observed in any of these trees. Table 20 gives the average dry weights of the



Fig. 12. Trees grown in leached and unleached saline soil. Tree in center grown in unleached second-foot soil from an orange grove; other two trees grown in leached second-foot soil.

trees at the end of the experiment, and represents the relative growth made by the several series. The best growth was produced in the second-foot soil. Figure 12 shows certain trees in the leached and unleached soils just prior to the termination of the experiment in December, 1922. The amounts of chlorin in the ash of certain trees are given in table 21, and supplement the data given in table 17.

TABLE 21  
PERCENTAGE OF CHLORIN IN THE ASH OF ORANGE TREES GROWN IN UNLEACHED SALINE SOIL

	Leaves	Shoots	Trunks	Roots	Rootlets
First foot, unleached.....			4.45	11.99	
Second foot, unleached.....	11.79	2.71	1.98	3.28	3.58

*B. Trees grown in the field.*

Samples of leaves and shoots were collected in April from mature orange trees which had been severely injured by the application of saline irrigation water. For purposes of comparison, samples of leaves and shoots were also taken from healthy trees of the same age in an adjoining grove which had received non-saline irrigation water. The water applied to the injured trees contained 480 p.p.m chlorin



Fig. 13. Young shoots from orange trees in the field showing severe injury following the use of saline irrigation water.

and 200 of sodium. Samples of the soil from this grove were collected at the time the leaves and shoots were taken from the trees. Analyses of the soil samples made by S. M. Brown showed the following amounts of chlorin: 1st foot, 229 p.p.m.; 2nd foot, 211 p.p.m.; 3d foot, 90 p.p.m.; and 4th foot, 99 p.p.m.

The shoots from the injured trees were of two kinds, young and old. There was a region below the tip of each young shoot (fig. 13) which was discolored and shrunken. The older shoots were more or less completely defoliated and were putting out weak, unhealthy secondary shoots, many of which fell after making very feeble growth.



The young leaves were often greatly reduced in size and showed discolored, blistered surfaces (fig. 14). Leaves which reached the usual size often had dead tips or margins. The old leaves which were collected in April had been produced the preceding year and were dead at the tips and margins (fig. 15). At the time they were collected, many of them were falling from the trees. Table 22 shows the



Fig. 14. Young leaves from shoots like those shown in figure 13. The surfaces of these leaves had a blistered appearance.

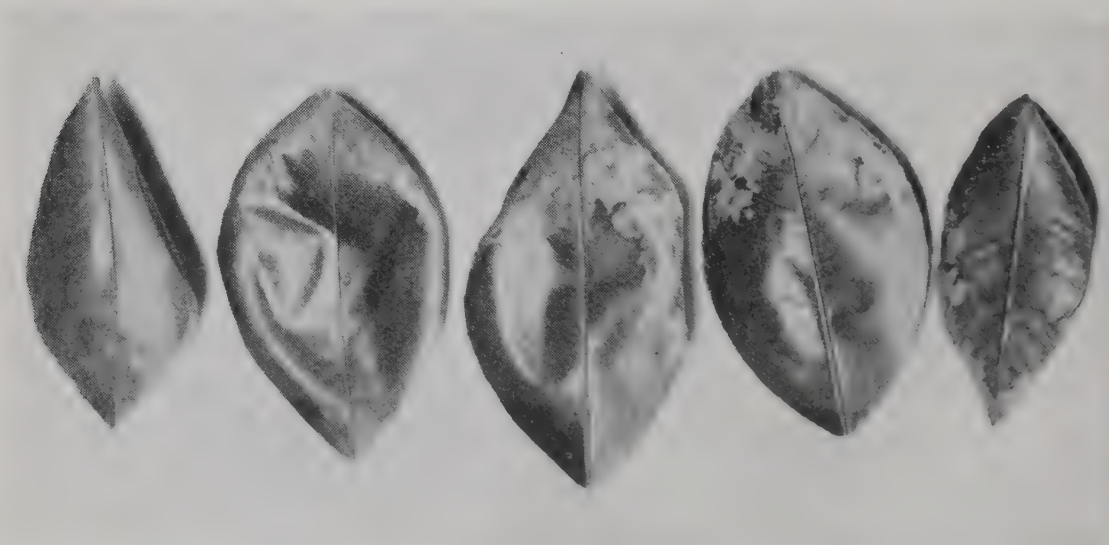


Fig. 15. Old leaves from orange trees which showed severe injury from saline irrigation water.

composition of the shoots and leaves. The very high percentage of chlorin in the shoots and leaves of the injured trees is one of the conspicuous features of this table. The percentage of chlorin in the young shoots and leaves was greater than that in the older organs. It may be well that only the organs which contained the smaller amounts of chlorin were able to survive. Another interesting feature of the analyses is the small percentage of sodium in the old injured leaves.

TABLE 22

THE COMPOSITION OF SHOOTS AND LEAVES OF ORANGE TREES IRRIGATED WITH SALINE AND NON-SALINE WATER

	Irrigated with saline water					Irrigated with non-saline water	
	Shoots		Leaves			Shoots	Leaves
	Young succulent shoots of the last cycle	Defoliated woody shoots of the preceding cycle	Young leaves showing injury	Old leaves still attached but injured	Old leaves fallen as a result of injury	Woody shoots of the preceding cycle	Old leaves not injured
Constituents of the ash:							
Na.....	5.78	2.12	4.71	1.28	1.19	1.19	2.23
K.....	18.45	5.85	19.41	3.55	2.75	6.92	5.22
Ca.....	18.91	29.00	17.05	30.80	32.10	31.30	32.00
Mg.....	4.52	4.38	3.96	4.02	4.29	2.62	2.23
Cl.....	22.64	7.23	22.70	15.57	14.45	0.14	0.33
SO <sub>4</sub> .....	1.98	4.46	2.40	3.58	3.73	2.43	3.11
PO <sub>4</sub> .....	6.04	5.81	7.83	2.18	1.48	4.48	1.99
Ash expressed as per cent of the dry matter.....	10.59	8.51	10.28	17.64	18.03	4.93	12.65

The percentage of ash in the dry matter of the woody shoots and of the old leaves on the injured trees was considerably greater than that of organs of the same age from healthy trees.

The results of these experiments and analyses extend our knowledge of the effects of sodium chlorid on orange trees. They agree in showing reduced growth of roots, shoots, and leaves. Eventually the trees suffer from premature defoliation. The analyses show that the Na and Cl content of injured shoots is greater than that of healthy shoots.

It is also shown that harmful amounts of sodium chlorid may be leached from a soil and that satisfactory growth conditions may be produced if suitable nutrient salts are added soon after the leaching.

## IX. THE EFFECT OF SODIUM BICARBONATE ON YOUNG ORANGE TREES

In the present studies, Valencia orange trees (*Citrus sinensis* Osbeck), were grown in sand cultures to which modified Hoagland's solution containing 1000 p.p.m sodium bicarbonate was added. The technique of the care of the cultures was the same as that previously described. Table 23 shows the concentration of the various ions in the culture solutions and the salts employed. The solution applied to trees 30-35 contained no calcium, while that of the other two series contained 159 p.p.m. The  $P_H$  of the solution applied to trees 30-35 was 7.55, while that of trees 38, 40 and 41 was 7.45. The trees were planted in the containers of sand on May 21, 1920, and were removed on September 20, 1921, having grown during the same period as trees 6-11, 12 and 16, 18-23, and 27 and 28, the data on which have already been reported in previous publications.<sup>17, 18</sup>

TABLE 23

COMPOSITION OF CULTURE SOLUTIONS EMPLOYED IN EXPERIMENTS ON THE EFFECTS OF BICARBONATES ON ORANGE TREES  
(Parts per million)

	Trees		
	1 and 2	30-35	38, 40, 41
Na.....	7	280	280
K.....	185	496	496
Mn.....	0.1	0.1	0.1
Ca.....	159	0	159
Mg.....	54	54	54
Fe.....	1	1	1
Cl.....	10	10	291
NO <sub>3</sub> .....	718	718	718
SO <sub>4</sub> .....	216	214	214
HCO <sub>3</sub> .....		725	725
PO <sub>4</sub> .....	105	105	105
Total concentration.....	1455.1	2603.1	3043.1

Salts employed in making the nutrient solution.

KNO <sub>3</sub>	KNO <sub>3</sub>	KNO <sub>3</sub>
MgSO <sub>4</sub> +7H <sub>2</sub> O	MgSO <sub>4</sub> +7H <sub>2</sub> O	MgSO <sub>4</sub> +7H <sub>2</sub> O
NaCl	NaCl	NaCl
Ca(NO <sub>3</sub> ) <sub>2</sub> +4H <sub>2</sub> O	KNO <sub>3</sub>	KNO <sub>3</sub>
KH <sub>2</sub> PO <sub>4</sub>	KH <sub>2</sub> PO <sub>4</sub>	KH <sub>2</sub> PO <sub>4</sub>
MnSO <sub>4</sub>	NaHCO <sub>3</sub>	NaHCO <sub>3</sub>
Ferric tartrate	MnSO <sub>4</sub>	CaCl <sub>2</sub> +2H <sub>2</sub> O
	Ferric tartrate	MnSO <sub>4</sub>
		Ferric tartrate



TABLE 24  
NUMBER OF LEAVES, DRY WEIGHT OF VARIOUS PORTIONS OF CITRUS TREES AND WATER REQUIREMENT

Tree	Number of leaves	Dry weight (dried at 60°-65° C.) in grams						Total culture solution added	Total distilled water added	Total drainage water	Trans- pira- tion	Water require- ment
		Leaves	Shoots	Trunk	Root	Rootlets	Total					
30 .....	52	4.5	7	58	47	9.5	126	Liters 184	Liters 49	Liters 145.5	Liters 87.5	690
31 .....	190	27	9.5	134	164	13	347.5	192	61	141.25	111.75	320
32 .....	81	8.5	14	92	103	8	225.5	196	47	138.35	104.65	460
33 .....	218	22	12.5	70	87	17	208.5	186	55	142.80	98.2	470
34 .....	93	19	13	51	55	13.5	151.5	178	41	136.60	82.4	540
35 .....	96	15	24	127	133	13	312	186	56	131.1	110.9	360
Average per tree (30-35) .....	122	16	13.3	88.7	98.2	12.3	228.5					
38 .....	385	54	26	86	84	39.7	289.7	208	93	109.9	191.1	660
40 .....	330	53	19.5	86	76	34	268.5	208	75	150.75	132.25	490
41 .....	425	84.5	33	80	145	31	373.5	236	75	125.08	185.92	500
Average per tree (38, 40, 41) .....	380	63.8	26.2	84	101.7	34.9	310.6					

TABLE 25  
EFFECTS OF SODIUM SALTS ON ORANGE TREES  
Number of leaves, dry weight, and transpiration of average trees of each series

Series	Concentration of ions in addition to that of the control	Number of leaves on average tree	Dry weight (dried at 60°-65° C.) in grams						Transpiration (liters)
			Leaves	Shoots	Trunk	Root	Rootlets	Total	
42-46	Ca of Hoagland's solution replaced by K.....	110	19	20.8	85.1	115	18.9	258.3	85.9
6-11	393 p.p.m. Na..... 607 p.p.m. Cl.....	97	15.6	12.8	91.7	78.9	12.1	211.2	74
18-23	323 p.p.m. Na..... 674 p.p.m. SO <sub>4</sub> .....	53	14.7	14.6	75.2	107.3	21.8	233.5	95.5
30-35	273 p.p.m. Na..... 725 p.p.m. HCO <sub>3</sub> .....	122	16	13.3	88.7	98.2	12.3	228.5	99.2
13 and 16	393 p.p.m. Na..... 159 p.p.m. Ca..... 888 p.p.m. Cl.....	498	95.2	35.2	104.2	106	46	386.6	126
27 and 28	323 p.p.m. Na..... 674 p.p.m. SO <sub>4</sub> ..... 159 p.p.m. Ca..... 281 p.p.m. Cl.....	749	173	86.5	139	132.5	124	655	183.1
38, 40 and 41	273 p.p.m. Na..... 725 p.p.m. HCO <sub>3</sub> ..... 159 p.p.m. Ca..... 281 p.p.m. Cl.....	380	63.8	26.2	84	101.7	34.9	310.6	169.8
1	Control .....	996	237.1	122.5	194.0	212	136	901.6	228

The data in table 24 show the number of leaves, dry weight, and transpiration of the trees in the two series. Table 25 gives a wider comparison of the composition of the solutions and the average number of leaves, dry weight and transpiration of average trees in these and other series to which sodium salts were added at the rate of 1000 p.p.m. It shows that the growth made by trees 30-35 was similar to that made by the other trees receiving no calcium. The trees grown with the



Diagram 3. Comparative effects of three sodium salts upon orange trees. Each salt was added to the culture solution at the rate of 1000 p.p.m. The shaded bars represent number of leaves on the trees, and the unshaded bars represent grams of dry weight of trees. A, complete nutrient solution; B, nutrient solution plus 1000 p.p.m. sodium sulfate; C, nutrient solution plus 1000 p.p.m. sodium chlorid; D, nutrient solution plus 1000 p.p.m. sodium bicarbonate.



additional sulfate (trees 27 and 28) grew better than trees which received the additional chlorin (trees 13 and 16) and these in turn grew better than trees which received bicarbonate (trees 38, 40 and 41). The comparative effects of three sodium salts upon the growth of orange trees are shown in diagram 3.

The effect of bicarbonate on walnut seedlings (table 6) was also more toxic than that of chlorid or sulfate in conjunction with sodium cations.



Fig. 16. Trees 34 and 35 at the end of fifteen months in sand cultures which had received modified nutrient solution lacking calcium and containing 1000 p.p.m. sodium bicarbonate.

Figure 16 shows the tops of trees 34 and 35 on August 25, 1921, after 15 months in cultures which received sodium bicarbonate but no calcium. Many of the leaves had fallen and most of those produced subsequently fell before attaining full size. Many of the shoots became leafless and died. Figure 16 also shows the recurved condition, typical of leaves grown in the absence of calcium (cf. 18, pl. 3, fig. 1). The trunks were still alive when the trees were removed from the containers. The trunk and root system are the last parts of an orange tree to die, under these conditions, due possibly to their retention of calcium and to the slower rate of metabolism in their tissues.



Fig. 17. Trees 38, 40 and 41 at the end of fifteen months in sand cultures which received complete nutrient solution plus 1000 p.p.m. sodium bicarbonate.



Fig. 18. Trees in sand cultures both of which received 1000 p.p.m. sodium bicarbonate. The tree on the left received no calcium, while the tree on the right received 159 p.p.m. calcium as calcium chlorid. The photograph was taken fifteen months after the experiment began.



Figure 17 shows the growth made by the tree tops when sodium bicarbonate was present along with calcium chlorid. The leaves were retained much longer than in the case where calcium was absent from the culture solution. Figure 18 contrasts the condition of the tree tops when calcium is present, with the defoliation that occurs when it is absent. The chlorotic leaves shown in figure 19 are typical of many which appeared on trees 30–35 after a year had passed. These leaves were similar to those which are frequently found on trees in groves where the soil contains bicarbonates and carbonates (Lipman,<sup>11</sup>).

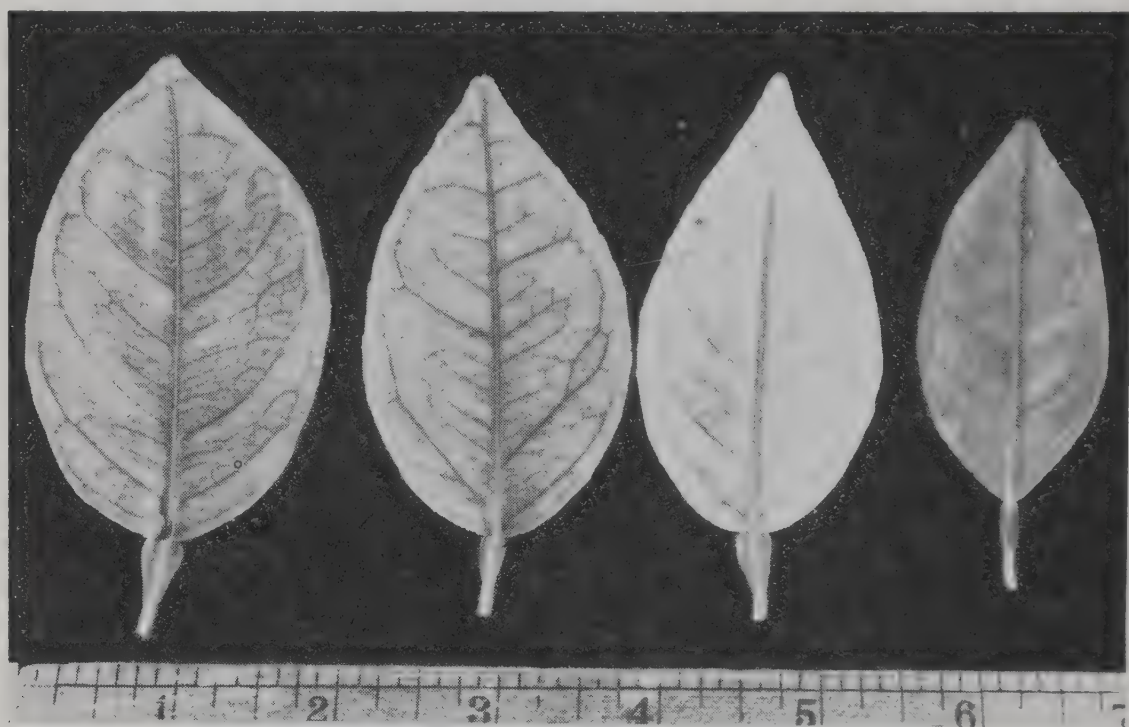


Fig. 19. Chlorotic leaves from trees which received sodium bicarbonate without calcium. Leaves like these never recovered. Scale of inches at the bottom of the picture.

When a condition of chlorosis like that shown by these leaves is attained, no recovery occurs, even though iron is added to the cultures at frequent intervals. The leaves of trees 38, 40 and 41, which received calcium chlorid in addition to sodium bicarbonate, showed no such chlorotic symptoms. The chlorotic condition shown in figure 19 is much more pronounced than that due to the absence of potassium as noted in one of our previous papers<sup>19</sup> (pl. 5, fig. 2), where recovery subsequently followed.

Figure 20 shows the root systems of trees 33 and 35 and of tree 1 which received Hoagland's nutrient solution. Figure 21 shows the root systems of trees 40 and 41 and of tree 1. The root systems of trees 30–35 were very poor, and contained many dead slimy rootlets.





Fig. 20. The effect of solutions containing sodium bicarbonate without calcium on the development of the root system of orange trees. No. 1 grew in a culture receiving a complete nutrient solution; Nos. 33 and 35 grew in cultures receiving nutrient solutions containing 1000 p.p.m. sodium bicarbonate but without calcium (compare fig. 21).

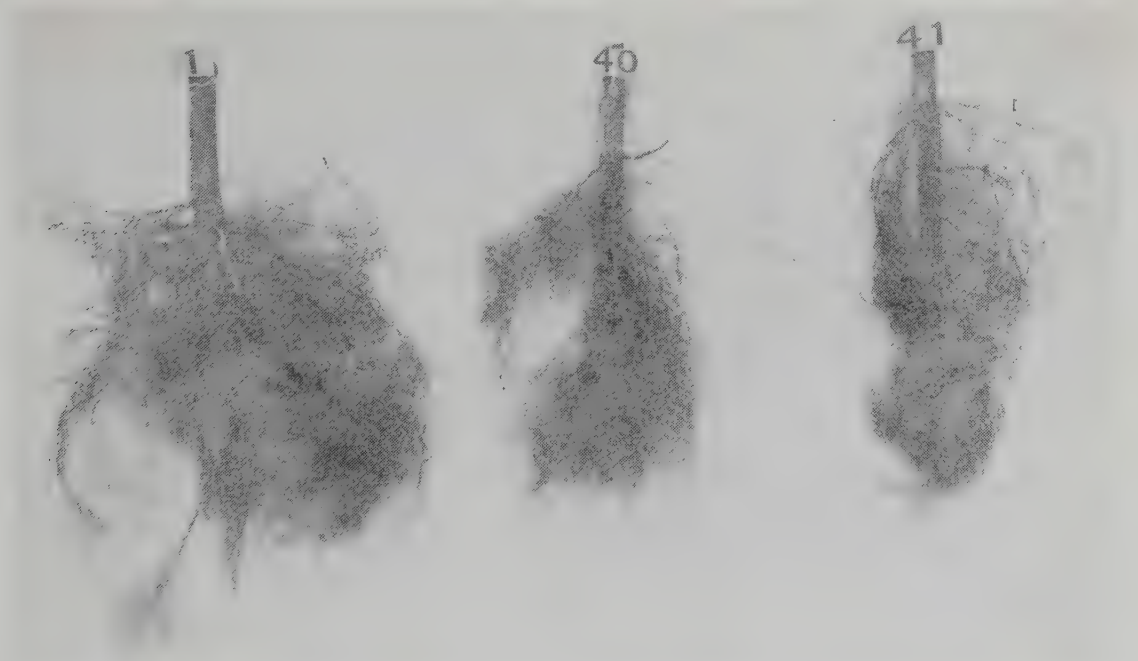


Fig. 21. The effect of solutions containing sodium bicarbonate plus calcium on the development of orange tree roots. No. 1 grew in a culture receiving a complete nutrient solution; Nos. 40 and 41 grew in cultures receiving a complete nutrient solution plus 1000 p.p.m. sodium bicarbonate (compare fig. 20).

The root systems of trees 38, 40 and 41 were small compared with those grown with Hoagland's complete solution, but contained few rotting or slimy rootlets.

Several determinations were made of the  $P_H$  of the drainage waters from cans 30-35 and 36-41. It was found to range from that of the culture solution applied to faint alkalinity to phenolphthalein, that is, from  $P_H$  7.5 to  $P_H$  8.5. This indicates that bicarbonates in sand cultures may be converted to some extent into carbonates. The alkalinity present in these sand cultures no doubt greatly reduced the amount of iron available for the rootlets as described in another paper,<sup>22</sup> and also greatly reduced the available supply of phosphate and calcium. The first 500 c.c. of percolate obtained from the cultures upon adding distilled water showed no appreciable change in nitrate from the initial concentration. The phosphate found in the drainage water of series 30-35 showed no change in concentration from that of the solution applied, while that found in the drainage water of series 36-41 showed a reduction to the low average value of 4 p.p.m. Some of the original culture solution for series 36-41 was left standing a few hours and the concentration of phosphate was reduced from 105 p.p.m. to 28 p.p.m.

We cannot ignore the fact, therefore, that one of the potent effects of high OH-ion concentration is to reduce the solubility of certain other ions in the culture solution. In studying the effects of an alkaline solution upon plants, we have to take into account not alone the effect of the hydroxyl-ion concentration upon the plant, but also its effect upon the solubility of the ions in the medium which bathes the rootlets.

Samples of the various portions of trees 30-35 and trees 38, 40 and 41 were analyzed and the results are given in table 26 as percentages of dry matter and of ash.

Total nitrogen and phosphorus were greatest in the poorest trees (30-35). The total sulfur was determined only in the leaves, and by comparison with the  $SO_4$  found in the ash it appears that an appreciable percentage of the total sulfur exists in the organic form. We find that the phosphorus can be largely accounted for as  $PO_4$  in the leaves and shoots, organic combinations of phosphorus being inappreciable in amount.

An important point in the data of table 26 is the very low percentage of sodium in the ash of the leaves and shoots, and the fact

TABLE 26  
EFFECTS OF BICARBONATE UPON THE COMPOSITION OF DIFFERENT PARTS OF VALENCIA ORANGE TREES

		(Expressed as per cent of dry matter)						(Expressed as per cent of ash)					
		Leaves		Shoots		Trunk		Roots		Rootlets *		Leaves	
		Trees		Trees		Trees		Trees		Trees		Trees	
		30-35	38, 40, 41	30-35	38, 40, 41	30-35	38, 40, 41	30-35	38, 40, 41	30-35	38, 40, 41	30-35	38, 40, 41
Total N	2.58	2.36	1.68	1.16	0.66	0.79	0.66	0.83	0.69	2.28	1.79		
Total S	0.13	0.23											
Total P	0.20	0.15	0.18	0.09	0.06	0.06	0.04	0.06	0.05	0.24	0.12		
Ash	16.23	19.20	9.58	8.81	4.08	3.61	2.83	3.02	3.02	8.62	11.29		
Na	0.12	0.13	0.06	0.07	0.24	0.12	0.31	0.20	0.31	0.46	0.56	0.75	0.63
K	7.88	8.17	4.04	2.92	0.80	0.79	0.39	0.39	0.52	2.45	2.71	48.50	42.55
Cl	0.05	0.14	0.02	0.13	0.01	0.05	0.31	0.31	0.31	0.33	0.90	0.29	0.70
Ca	0.33	1.18	0.41	1.06	0.64	0.61	0.42	0.55	0.55	0.54	1.39	1.99	6.14
Mg	0.30	0.31	0.27	0.31	0.10	0.08	0.09	0.05	0.05	0.41	0.39	1.80	1.61
SO <sub>4</sub>												2.28	2.28
PO <sub>4</sub>												3.70	2.28

\* Rootlets calculated to silica-free basis.



that the trunk, root and rootlets contain greater percentages of sodium than the leaves and shoots. Thus far in our studies upon absorption and distribution of elements throughout citrus trees, we have always found a lower percentage of sodium in the ash of leaves and shoots than in the other portions of the tree, and the percentage of sodium has thus far never been found to be relatively high.

All parts of the trees are relatively rich in potassium, the percentage decreasing as we pass from the leaves to the rootlets where there is an increase over that found in the root. The cells of orange trees absorb large amounts of potassium when it is available. In this respect they resemble animal cells, which may accumulate large amounts of potassium, as has been shown by Mitchell and Wilson<sup>13</sup> and others. The amounts of chlorin in the two sets of trees correspond in a way to the concentrations of chlorid furnished them. The repeated losses of older leaves may account for the low Cl content of the leaves, especially in trees 30-35.

The absence of calcium from the culture solution of series 30-35 is reflected most conspicuously in the leaves, shoots, and rootlets, indicating that the calcium of the trunk and root is relatively immobile. Lee<sup>10</sup> has reported the occurrence of mottle-leaf on citrus in the Philippine Islands which is correlated with the species of rootstock employed. It is desirable that studies be made of the calcium requirement and the calcium-transporting power of these rootstocks. At present we cannot say whether a deficiency of calcium in the leaves is due to low calcium mobility or low conductivity through the woody portions of the tree, or whether the alkaline medium which bathes the rootlets has changed the permeability of the cells or has brought about the almost complete precipitation of certain essential ions from the nutrient solution, thereby preventing the plant from obtaining an adequate supply of such ions.

In addition to chlorosis in series 30-35 there was also evidence of mottling. As has been stated, however, the leaves on trees 30-35 fell at so early a stage that it was not possible to follow their condition for any considerable length of time. No initial stages of mottle-leaf were evident in series 36-41, though growth was greatly reduced, the frequent renewal of solution, no doubt, having supplied the trees with sufficient calcium and other ions to maintain themselves. We doubt very much if mottle-leaf is the direct result of an alkaline nutrient

medium. If mottling is found to be present where the culture medium is alkaline, it is possible that it is the result of secondary causes induced by high alkalinity.

#### X. RELATIONS BETWEEN CALCIUM DEFICIENCY AND CHLOROSIS

In the course of these experiments on the effects of certain salts on the physiology of the orange tree, we have accumulated evidence of a peculiarly close relationship between calcium deficiency and chlorosis. The relation of iron deficiency to chlorosis is well known and it has been shown that a concentration of OH ions which greatly reduces the solubility of iron will induce chlorosis in plants. Several other causes of chlorosis are known so that it is evident that more than one cause may produce this effect. Our object is to call attention to the way in which a deficiency of calcium may cause chlorosis.

We have previously described<sup>18</sup> the chlorotic condition of orange leaves where the trees received a culture solution lacking calcium, but containing sodium salts in considerable quantities. It was shown that such trees lost their leaves prematurely and produced a succession of small chlorotic leaves which were likewise prematurely lost. It is not to be concluded from these observations that the absence of calcium from the culture solution was the sole cause of the chlorosis.

Since the appearance of the publication just cited we have obtained additional data from trees 60-67 grown in sand cultures. The trees were planted in May, 1921, and received a culture solution containing high NaCl and low CaCl<sub>2</sub> in the ratio of 98 NaCl: 2 CaCl<sub>2</sub>. The composition of the solution was the same as that applied to trees 6-11 in the publication just cited, except that it contained the small amount of CaCl<sub>2</sub>.

For a time trees 60-67 produced good foliage and shoots, but during the winter period of comparative dormancy all the leaves were shed from the trees and many of the shoots died. From the time this symptom appeared the trees were given a complete nutrient solution containing double the usual quantity of salts but lacking the excess NaCl formerly included. New leaves were produced the following spring, but none survived long except those on short shoots arising from the lower portion of the trunk. Many of these leaves were shed and were followed by other crops of leaves. Most of the leaves on the short shoots reached full size, but very few had the normal green color.



Some of them were entirely albescent, in some the veins were green though the tissue between them was pale green or yellow (fig. 22).

When the trees were removed at the termination of the experiment, we found that the greater part of the root system was dead. It appeared that the trees had first produced a fair root system, but that they reached a limit when the small amount of Ca at their disposal was inadequate. The terminal parts of many of the older roots died and then new roots were produced from the living region near the primary root, but many of the second roots likewise died back for

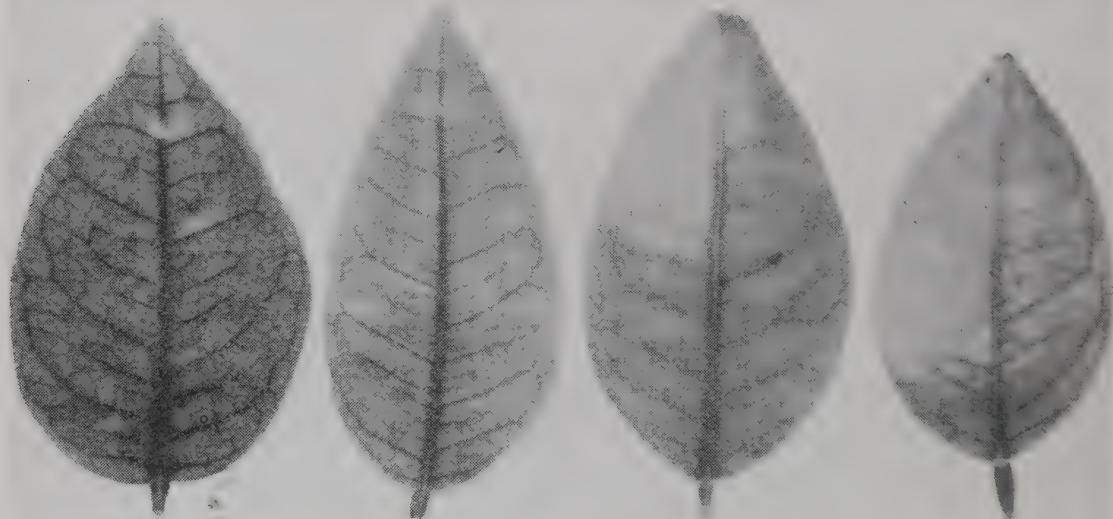


Fig. 22. Chlorotic leaves from orange trees in cultures which received a small amount of calcium in comparison with sodium.

a distance from the apical end. The failure of the trees to produce new roots after the addition of a complete nutrient solution seems to be related to the absence of green foliage. The lack of a suitable supply of carbohydrates may have been an important factor in producing this condition.

We have found chlorotic trees in cultures where the calcium supply was not limited but where faulty drainage caused the roots to die. In such cases the leaves were not shed, and the trees recovered after suitable drainage was provided. The chlorotic condition of leaves generally appears when orange roots die and decay to any considerable extent.

We found that the addition of sodium bicarbonate to the culture solution restricted the growth of roots and shoots and caused a definite



type of chlorosis where calcium was deficient. It is more than likely that the alkalinity of the culture solutions tended to precipitate certain ions, though the injury observed was not due entirely to the deficiency of nutrient ions.

## XI. INTERRELATIONS BETWEEN POTASSIUM AND CALCIUM

There is an increasing amount of evidence of an opposite relationship between the proportions of potassium and calcium in the citrus tree. Kelley and Cummins<sup>8</sup> have reported on the relationships between these ions in healthy and mottled leaves of certain species of citrus growing in the field. They showed that citrus leaves affected with "mottle-leaf" contain more K and less Ca than normal leaves of the same age. The writers<sup>18</sup> have published further evidence obtained from young orange trees grown in sand cultures under controlled conditions. The ash of trees grown in control cultures contained approximately 25 per cent K and 19 per cent Ca. The ash of trees to which calcium-deficient solutions were applied contained approximately 50 per cent K and 1 per cent Ca. Conversely, the ash of trees to which potassium-deficient solutions were applied contained approximately 30 per cent Ca and 1 per cent K. The writers have found other instances which indicate that the relations between K and Ca in the citrus tree stand in intimate connection with growth and other physiological processes.

Four series of trees in sand cultures (table 27) were grown under conditions previously described. The control series JJ (trees 105-110) received Hoagland's nutrient solution, double strength; series KK (trees 42-46) received nutrient solution (single strength) in which K was substituted for Ca; series LL (trees 111-116) received nutrient solution containing 311 p.p.m. additional K as KCl; series MM (trees 123-129) was like the series KK with the addition of 15 p.p.m. Ca as  $\text{Ca}(\text{NO}_3)_2$ .

The trees in the control series JJ made very satisfactory growth and showed no indications of mottle-leaf at any time during the course of the experiment. The leaves were dark green; the shoots and roots were well developed. The trees in series KK made very restricted growth. Their leaves showed the effects of calcium deficiency previously described, viz., premature abscission, chlorosis accompanied by small spots of dead tissue, and abnormal curling. Their roots grew

TABLE 27

GROWTH AND COMPOSITION OF YOUNG VALENCIA ORANGE TREES IN RELATION TO POTASSIUM AND CALCIUM

Series	JJ	KK	LL	MM
Trees.....	105-110	42-46	111-116	123-129
Culture solution contained	370 p.p.m. K 318 p.p.m. Ca 108 p.p.m. Mg 20 p.p.m. Cl	496 p.p.m. K 0 p.p.m. Ca 54 p.p.m. Mg 10 p.p.m. Cl	496 p.p.m. K 159 p.p.m. Ca 54 p.p.m. Mg 291 p.p.m. Cl	496 p.p.m. K 15 p.p.m. Ca 54 p.p.m. Mg 10 p.p.m. Cl
Osmotic pressure of cul- ture solution (atmos- pheres).....	1.452	.708	1.242	.724
Average number of leaves per tree.....	1,351	110	1,084	580
Average dry weight per tree (grams).....	1,417	258	1,080	1,078
Average units of water transpired per unit of dry matter.....	319	332	343	356
	K Ca Mg Cl	K Ca Mg Cl	K Ca Mg Cl	K Ca Mg Cl
Per cent in the ash:				
Leaves.....	18.23 20.88 1.85 .30	50.04 1.28 1.16 .10	30.06 13.15 1.26 3.39	41.38 6.18 1.80 .33
Shoots.....	14.89 22.95 2.40 .30	44.86 2.98 1.79 Trace.	24.87 16.64 1.93 4.42	39.36 5.58 3.24 .28
Trunks.....	13.97 22.70 1.68 .39	26.65 16.50 2.81 Trace.	20.34 19.12 1.46 2.32	25.14 15.38 2.81 .25
Roots.....	15.59 17.73 2.35 .38	24.11 13.49 3.34 Trace.	21.68 13.74 2.15 2.05	23.29 11.39 3.45 .21
Rootlets..... (silica free basis)	28.33 7.80 3.71 1.57	36.71 4.55 5.95 1.55	33.91 5.19 2.67 9.44	35.48 2.60 4.48 1.25

poorly and died prematurely. These conditions are reflected in the data (table 27) on the number of leaves and dry weight of trees.

The trees in series LL made good growth and were quite similar to those in the control series. Their foliage was different from that of the controls in that many of the young leaves had small yellow areas in the marginal tissue between the larger veins (fig. 23). The remainder of the leaf tissue was dark green. The yellow areas were covered subsequently with small brown papillae which were quite



Fig. 23. Orange leaves from series LL which received nutrient solution plus potassium chlorid. The marginal chlorotic spots show numerous brown papillae.

characteristic of this type of injury. As time went on the yellow areas became pale green, but they never attained the normal green color of the rest of the leaf. These affected leaves had no tendency to premature abscission. The roots of these trees compared very favorably in size and development with those of the control trees.

Trees in series MM made fairly good growth for a time, but later their leaves began to show yellow circular spots in the tissue between veins (fig. 24). These spots soon became confluent and involved much of the leaf area. Most of the leaves affected in this way fell from the trees prematurely and were followed by other crops of leaves which were smaller, were chlorotic about the margins, and bore brown



papillae on the chlorotic area (fig. 25). While the symptoms resembled those noted for leaves in series LL, they were generally indicative of more severe injury. We noted also that the injury was more severe on leaves produced during the hot summer months. Many of the depauperate leaves produced in the succeeding crops had no green tissue except near the veins and midrib (fig. 26) and therefore resemble closely the condition known as "mottle-leaf" or "frenching." The photomicrograph of a leaf section (fig. 27) shows something of the



Fig. 26. Orange leaves from series MM showing the mottled depauperate condition finally attained.

nature of the papillae on the affected leaves. The illustration shows two elevations covered with a thick dense layer. The elevations appear to have been produced by a proliferation of cells of the palisade layers. The thick superficial layer was seldom ruptured and we found no indication of parasites in the affected areas. Only a portion of the leaves on the trees in series MM were affected in the manner described; the remainder made very satisfactory growth without showing any of the symptoms noted.

Fig. 28 shows a representative tree from each of the two series LL and MM. It will be seen that there was more defoliation in the case of MM where only 15 p.p.m. calcium was supplied to the culture than in the case of LL where 159 p.p.m. calcium was supplied.





Fig. 24. Orange leaves from series MM which received nutrient solution deficient in calcium plus potassium chlorid. These leaves show an early stage of injury which resembles mottling.



Fig. 25. Orange leaves from series MM showing more advanced stage of injury. The yellow areas are covered with small brown papillae.





Fig. 29. Orange leaves from trees which received a magnesium-free nutrient solution for two years.





Fig. 27. Photomicrograph of a section of an orange leaf bearing papillae like those shown in figures 23 and 25.

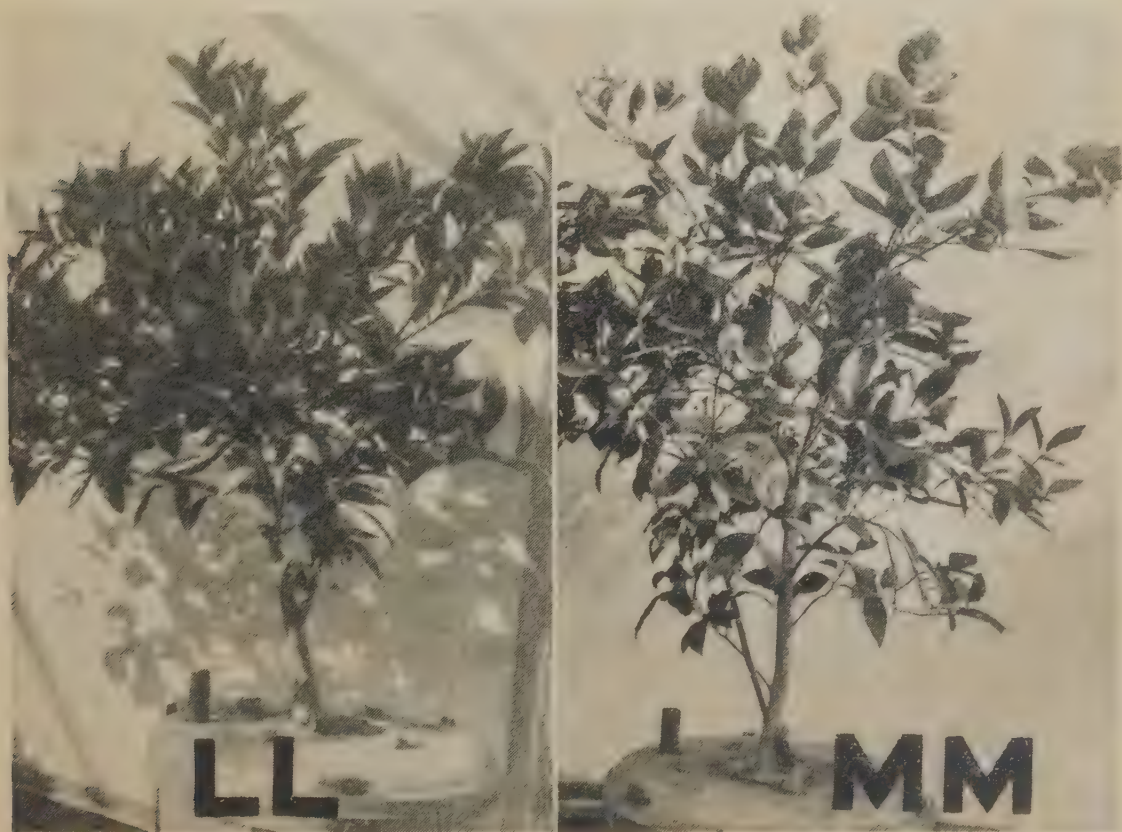


Fig. 28. Representative trees from series LL and MM. The partial defoliation of the trees from series MM is apparent.



The root system of trees in series JJ and LL was large and well developed, but in MM the roots made restricted growth and contained many rotten rootlets.

The percentage of K (table 27) was generally higher in the ash of leaves and rootlets (except when roots died) than in other parts of the trees. The ash of trunks and roots had considerably smaller percentages of K than other parts of the trees. The leaves and shoots of the trees in series KK were notably high in their K content, although the trees received the same concentration of K in the culture solution as trees in series LL and MM. The K content of these trees shows an inverse relationship to Ca content similar to that which has been discussed previously. The parts of the trees which showed the most serious effects were those which had the highest per cent of K in their ash.

The Ca content of the trees corresponded in a qualitative way with the Ca content of the culture solutions, that is, the trees in series KK which received no Ca contained the least calcium, and trees in series JJ which received a solution containing 318 p.p.m. Ca contained most. The trunks and roots in most cases contained more Ca than other parts of the trees.

The variations in the per cent of Mg in the ash were small and bore no apparent relation to the condition of the trees.

The Cl content of the ash was, in no case, high enough to be responsible for any of the injurious effects observed. In fact, the trees in series KK and MM which showed injury contained negligible amounts of Cl.

Analyses of the affected leaves from series MM showed minor differences in the content of certain ash constituents. Their composition was:

	Per cent of ash	Na	K	Ca	Mg	Cl	SO <sub>4</sub>	PO <sub>4</sub>
Affected .....	13.43	9.77	45.27	4.00	1.66	.32	3.86	3.54
Healthy .....	13.58	9.30	41.38	6.18	1.80	.33	3.79	3.35

The amounts of the ions are expressed as percentages of the ash. The leaves were selected as closely as possible for uniform age. The differences in composition, while small, agree with those found by Kelley and Cummins<sup>s</sup> in the case of normal and mottled leaves.

TABLE 28  
GROWTH AND COMPOSITION OF VALENCIA ORANGE TREES IN RELATION TO MAGNESIUM

Series	NN	OO
Culture solutions contained.....	14 p.p.m. Na 108 p.p.m. Mg 210 p.p.m. PO <sub>4</sub>	110 p.p.m. Na 0 p.p.m. Mg 105 p.p.m. PO <sub>4</sub>
Osmotic pressure of solution.....	1 452 atmospheres	.965 atmospheres
Number of leaves per tree.....	1,351	1,379
Dry weight per tree.....	1,417	1,486
Units of water transpired per unit of dry matter.....	319	317
	NaKCaMgSO <sub>4</sub> PO <sub>4</sub>	NaKMgCaSO <sub>4</sub> PO <sub>4</sub>
Per cent in the ash:		
Leaves.....	4.5818.2320.881.854.394.70	5.9820.9419.57.574.073.78
Shoots.....	3.2914.8922.952.404.867.77	4.0214.7024.66.622.815.02
Trunks.....	3.4213.9722.701.683.756.82	5.1615.3522.251.156.57
Roots.....	3.7115.5917.732.352.688.15	6.6812.4317.221.176.50
Rootlets (silica-free basis).....	7.0928.337.803.7112.5212.05	12.1922.096.10.8012.297.51

The results of this experiment bring out some facts concerning the relations between K and Ca which seem worthy of notice. The marked injury shown by trees in series KK was so similar to what has been observed in other cases that it can be ascribed principally to Ca starvation. The yellow spots on the leaves, the premature abscission, and the gelatinous dead roots were characteristic of Ca starvation. The effects of Ca deficiency were so great as to make it difficult to distinguish any other effects.

In series LL the injurious effects were insignificant or at least transient; on the contrary, the injurious effects noted in series MM were obvious and became more severe as time went on. It would appear that, in the latter case, the amount of Ca furnished the trees was sufficient to prevent the most acute results of Ca starvation though it did not prevent the trees from taking up large amounts of K. In the case of series LL, the larger concentration of Ca furnished, changed the proportion of K and Ca in the ash, and allowed the trees to make satisfactory growth.

Attention may be called to the fact that, in a large series of experiments upon the effects of various salts upon orange trees, the writers have not hitherto obtained results which resembled as closely the condition known as "mottle-leaf" as those here reported. It appears to us that the development of mottle-leaf is related to certain conditions in which the amount of K present greatly exceeds that of Ca. This is not to imply that we think there is a particular proportion between K and Ca which causes this condition; on the contrary, there may be a rather wide range of conditions which produce a similar result. The concentration of monovalent ions may be in excess of that of Ca without producing effects on the trees such as here described. For example, another series which received a culture solution containing concentrations in parts per million as follows, K 185, Na 400, Ca 159, and Cl 617, showed none of the symptoms of mottling found in trees of series LL nor of mottling and abscission found in trees MM.

The H-ion concentration of the sap of healthy mature leaves from series LL and MM showed no significant difference. The former had a  $P_H$  of 5.82 and the latter 5.92, as determined electrometrically. Their titration curves were practically identical.



## XII. EFFECTS OF MAGNESIUM DEFICIENCY ON YOUNG ORANGE TREES

Six trees were planted in the sand cultures April 5, 1921, and removed January 5, 1923. The care of the cultures was like that described in the preceding paragraphs. The composition of the magnesium-free culture solution in parts per million of ions was

Na	K	Ca	Mg	Cl	NO <sub>3</sub>	SO <sub>4</sub>	PO <sub>4</sub>	Fe	Mn
110	185	159	0	10	718	214	105	1	0.1

giving a total concentration of 1502.1 parts per million. The control series NN grown simultaneously received double-strength nutrient solution.

The trees in the magnesium-free series 00 grew well and attained a development at the end of the period mentioned equal in all respects to those in the control series NN (table 28). The only significant difference was a peculiar chlorotic stripe on the older leaves and this character did not appear until the experiment had been in progress about a year. The nature and development of this chlorotic condition are shown in fig. 29. Between the larger veins in the initial stages, there was a series of yellow areas situated a short distance on either side of the midrib of the leaf. As these yellow areas enlarge they coalesced to form a well-defined stripe in the middle of the leaf and gradually covered the veins and midrib. The bleaching was most pronounced at the base of the leaf. In course of time the chlorotic area extended toward the margin of the leaf as shown at the right of fig. 29, and eventually many leaves were entirely chlorotic. We noticed no premature abscission of these chlorotic leaves. Only part of the leaves on any tree showed this condition and they were usually in the lower, interior part of the tree.

The composition of various parts of the trees (table 28) shows few significant differences when compared with that of the control trees. The principal difference was in the Mg content, as one might expect. The lowest content of Mg was found in the ash of leaves, shoots, and rootlets. We have previously shown<sup>23</sup> that the Mg of the trunk and root of an orange tree is somewhat less soluble than that of other parts of the tree, and it is possible that the original content of Mg in these trees had not been entirely reduced by transfer to the more actively growing portions of the tree.

Attention may be called to the significance of this fact in the nutrition of trees. Although the tree when planted contained about 2 per cent of Mg in its ash, this small amount was sufficient to maintain good growth and no untoward effects of any kind were observed during the first year. Somewhat similar results were noted when orange trees were deprived of potassium, but, on the contrary, injury was soon apparent when trees were deprived of calcium.<sup>19</sup> Trees deprived of Mg showed no striking changes in their content of other ions, although there was some reduction in the amount of  $\text{PO}_4$  in the ash of the rootlets.

In view of these results we may assume that a relatively small amount of magnesium is adequate for satisfactory growth and chlorophyll production. This assumption is supported by the fact that good growth is obtained when the trees are given a nutrient solution containing Mg in a concentration of 54 parts per million.

## SUMMARY

The data presented in the preceding pages give some of the results obtained from several series of experiments with seedlings and young trees. They are designed to give some information on the phenomena of absorption of ions and the consequent effects upon growth. Walnut seedlings absorbed sulphate in increased amounts when the concentration of that ion in the culture solution was increased, although the absorption was by no means parallel to the concentration. Seedlings supplied with a culture solution containing 1200 p.p.m. sulphate made very good growth, but, in higher concentrations growth was restricted and leaves were killed at the margins.

Concentrations of nitrate as high as 1600 p.p.m., furnished as sodium nitrate, had no detrimental effects on the growth of walnut seedlings, and higher concentrations only had a retarding influence upon the growth of tops. An initial concentration of 2700 p.p.m. nitrate produced injury to the margins of walnut leaflets similar to that produced by a concentration of 1500 p.p.m. chlorin as NaCl. A comparison of the growth attained in equi-molecular concentrations of sodium nitrate and sodium sulfate indicated that the latter is the less favorable.



Walnut and orange trees absorb chlorin readily and their growth is characteristically affected by amounts which would be harmless in the case of certain other anions. The roots of these seedlings usually contain more chlorin than the tops, though the leaves of orange trees may contain as high as 22 per cent chlorin in their ash. Between  $P_H$  6.0 and 9.0 the reaction of the culture solution seemed to have little influence upon the amount of chlorin in the ash of walnut seedlings. Rough-lemon seedlings grown in a similar range of  $P_H$  values had slightly more chlorin in the ash of tops at  $P_H$  9.0 than at lower values. The smallest percentage of chlorin in the ash of their roots was found in plants grown at  $P_H$  7.0, and the amounts in roots grown at  $P_H$  6.0, 8.0, and 9.0 were not significantly different.

Young orange trees grown in soil cultures were seriously injured by the application of irrigation water containing 880 p.p.m. chlorin (1500 p.p.m. NaCl). The leaves turned yellow and showed dead margins which are typical of salt injury. The chlorin content of the injured leaves was very high, and there was no evidence that chlorin migrates from the leaves prior to abscission, although it was soluble in water.

Soils containing harmful amounts of sodium chlorid were leached under controlled conditions and their salt content was reduced to a point which permitted the trees to make satisfactory growth, although it was necessary to add nutrient salts.

A concentration of 1000 p.p.m. of sodium bicarbonate restricts the growth of walnut roots and often prevents completely the development of the epicotyls. The application to young orange trees of culture solution deficient in calcium and containing sodium bicarbonate, retarded the growth of the tops and eventually killed the roots. The orange leaves first showed abnormal curling and translucent spots, followed by premature abscission. Many of the leaves developed chlorosis.

The injurious effects of high concentrations of potassium ions were not evident until considerable time had passed. Walnut seedlings in culture solutions containing 1700 p.p.m. potassium made good growth for a time, but eventually the foliage turned yellow and the roots ceased to grow. Young orange trees in sand cultures which received solutions containing 500 p.p.m. potassium grew well for the first year, but eventually their leaves developed characteristic yellow spots on which brown papillae appeared. Many of the depauperate



leaves subsequently produced resembled mottled leaves. These untoward symptoms were more severe in a series of cultures where the amount of calcium furnished was very small. The leaves and rootlets showed most severe injury from this cause and their ash was richer in potassium.

The data show that sodium is not severely toxic unless the concentration is fairly high. When a sodium salt was added to a complete nutrient solution the trees were not severely injured except in cases where concentration as such became a factor. The growth of the epicotyl of walnut seedlings was retarded by sodium salts more than the growth of roots; however, the roots were characteristically injured later.

Walnut seedlings made thrifty growth in solutions of calcium chlorid, and very restricted growth in equi-molecular solutions of sodium chlorid. Strong concentrations of chlorin as calcium chlorid were somewhat less toxic to rough-lemon seedlings than equivalent concentrations as sodium chlorid. When calcium was furnished in sub-minimal amounts, the roots of young orange trees died and the foliage was chlorotic. The ash of the trunk and the main root of the orange trees was richer in calcium than other parts of the tree, though in certain cases the older leaves were very rich in calcium. Additional evidence may be gathered from these experiments to support the idea that the absorption of calcium and potassium presents a sort of antithesis in the function of the citrus tree. If the amount of one element is high in the ash, the amount of the other will be low. The roots of walnut seedlings are extremely sensitive and are quickly injured in calcium-free solutions.

Culture solutions whose reaction was on the alkaline side of the neutral point were not detrimental to the growth of rough-lemon seedlings; in fact, they made more growth at  $P_H$  8.0 than at  $P_H$  6.0 or lower. Citrus and walnut seedlings grown at  $P_H$  values of 7.0, or higher, contained as much chlorin in the ash of their tops as those grown at lower  $P_H$  values. The  $P_H$  values of the sap expressed from walnut seedlings were quite uniform regardless of the reaction of the solution in which they were grown.

A concentration of 1500 p.p.m. sodium sulfate caused no apparent injury to walnut seedlings, but at a concentration of 3000 to 6000 p.p.m. sodium sulfate the growth of the seedlings was progressively

retarded. Increasing concentrations of sodium nitrate had somewhat the same effect.

It is very difficult to anticipate the effects of a given concentration of an ion in the culture solution upon the amount of that ion found in the plant, although, in a general way the per cent found in the plant reflects the concentration in the culture solution. The data show, however, that the variations are by no means proportional.

On account of its mobility in the plant we might expect to find chlorin rather uniformly distributed, but we find that it has a tendency to accumulate in the roots of orange trees and in the epicotyls of walnut seedlings.

The effect of a very low concentration of magnesium on orange trees was mainly evident in a peculiar type of chlorosis in which only the tissue along the midrib of the leaf was involved. In spite of a low content of magnesium in the ash of the trees, there was no evidence of any profound physiological disturbance.

The effects of sodium chlorid on trees growing in soils under controlled conditions throw some light upon certain conditions often seen in the field. The restricted growth of roots and shoots, and the premature abscission of leaves agree with the analyses which show an increased chlorin content of those members. The conditions for growth in saline soil were greatly improved as a result of leaching the cultures while the trees were growing in them.

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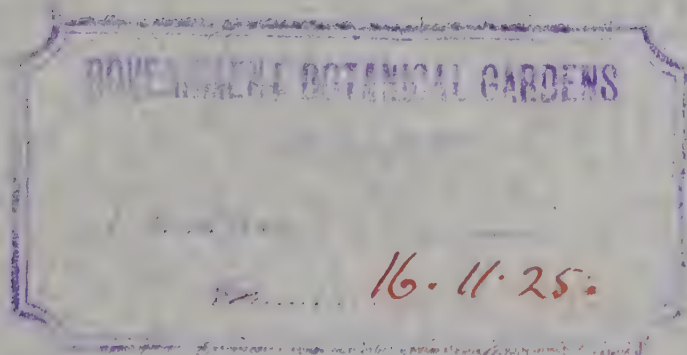
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# FACTORS INFLUENCING THE RATE OF GERMINATION OF THE SEED OF ASPARAGUS OFFICINALIS

BY

H. A. BORTHWICK



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## I. INTRODUCTION

Asparagus seed under average field conditions germinates slowly. In the Delta Region of the Sacramento River, which is the largest asparagus growing section in the United States, it is usually from two to six weeks before the seedling appears above the surface. This variation is due chiefly to differences in the temperature and moisture of the soil and in the depth of planting. Seed planted early is particularly slow in coming through the soil on account of the low

soil temperatures which prevail during the early part of the season. Even at these low temperatures, however, weeds may soon cover the ground and obscure the rows, making cultivation difficult. It has, in fact, been the custom in some asparagus growing sections to plant such quick-growing plants as radishes in the rows of asparagus so that the grower may see the rows before the asparagus appears and cultivate between them to destroy weeds.

No record has been found of any carefully controlled experiments to determine the influence of physical and chemical treatments upon the germination of asparagus seed. Hexamer<sup>1</sup> reports that "soaking the seed in lukewarm water for twenty-four hours before planting will hasten germination." Among growers there seems to be a diversity of opinion as to the value of soaking asparagus seed. Soaking asparagus seed before planting is practiced by a number of growers who assert that the plants come up a week or more ahead of those from unsoaked seed. Other growers maintain that they can see no difference in the rate of germination of soaked and unsoaked seed. Treatments other than soaking in water seem not to have been tried at all or at least not successfully, as no records have been found.

It is the purpose of this investigation to ascertain whether the period between the planting of asparagus seed and the appearance of seedlings above ground may be shortened by soaking in water. The temperature of the water, and the time of soaking are the factors involved.

Work is now in progress having to do with the influence of various physical and chemical treatments upon the germination of asparagus seed.

## II. LITERATURE

Numerous records are found in the literature having to do with the effect of soaking in water on germination of the seeds of various plants. A survey of such records shows that considerable variation exists in the results obtained. First, the seeds of different plants soaked in water under identical conditions may give entirely different germination results. Kidd and West<sup>4</sup> found that broad beans gave a better germination as a result of soaking, whereas a similar treatment decreased the germination of dwarf beans. Coupin<sup>6</sup> shows that many kinds of seeds are killed by soaking two weeks or even less but



that asparagus seed may survive 20 weeks' soaking. Second, seeds of the same kind of plant give markedly different germination results when the conditions under which they are soaked are varied. Coupin<sup>6</sup> finds that asparagus seed remains alive only 75 days in water which is changed frequently, but 145 days in unchanged water. On the other hand, he finds that beets will remain alive five times as long if the water is changed every day as they will if left in the same water the entire time.

Wollny<sup>3</sup> and Kraus<sup>2</sup> find that the amount of water in which certain seeds are soaked has much to do with their rate of germination.

These few brief references to the literature make obvious the fact that it is unsafe to predict the effect of soaking treatments on any kind of seed from results of similar treatments obtained with some other kind of seed. Moreover, the importance of controlling and describing the conditions under which the soaking treatments were carried out is emphasized.

### III. METHODS

*Source of Seed.*—The seed used in these experiments was obtained from the California Packing Corporation. It was grown on their ranch on Ryer Island in the Lower Sacramento Valley. Most of the experiments were made with two year old seed although some one year old seed was also used. Control experiments, however, showed no difference in germination of one and two year old seed.

Seeds soaked in water, in most cases, were soaked in uncorked bottles containing 100 cc. of water. The bottles were of such diameter that there were never more than two layers of seeds on the bottom. The seeds were covered by approximately five centimeters of water. They were placed in ovens, the temperatures of which did not fluctuate more than one degree centigrade. The duration of the soaking was accurately timed. This method of soaking is not in accord with the method adopted by Wollny,<sup>3</sup> and certain other workers who soaked their seed in as small an amount of water as possible in order to prevent excessive exosmosis of soluble food reserves. Wollny records that a reduction in germination and vigor of many of the seeds with which he worked, followed soaking in excessive amounts of water. I could find no such effect in the case of asparagus seed. For



this reason no attempt was made to keep the volume of water constantly small in the various experiments. It is of interest in this connection to note that Coupin<sup>6</sup> found that asparagus was able to withstand more than twice as much soaking as any other seed of twenty-three kinds he studied, provided no fresh water was added throughout the experiment.

*Germination Tests.*—Germination tests were made in the laboratory and in the field. In the laboratory tests, samples of 100 seeds each were germinated on cloth germinators made of Canton flannel strips 8 inches by 32 inches which by folding twice gave an 8 × 8 inch germinator four layers thick. The seeds had two layers above and two below. These germinators were placed in a constant temperature chamber in piles of not more than three each. Where the temperature of the germination chamber was other than 30° C., a note of it is made in the text. The germinators were kept moist by sprinkling once or twice a day. In the field tests, samples of 100 or 200 seeds were used. After treatment they were planted in rows about 2 inches deep.

The period of germination was reckoned from the time the seed was planted in the field or placed in the germinators until the first evidence of germination was visible. This in the laboratory tests was at the rupture of the seed coat—in the field at the emergence of the growing tip of the seedling. The field germination periods were, therefore, longer than corresponding laboratory germination periods because the shoots had to elongate at least two inches before they could be seen.

*Water Intake Determinations.*—Before water intake determinations were made, the seeds were carefully examined and all cracked and shriveled seeds discarded. The good seeds were then weighed in the air-dry condition. After various periods of soaking, the seeds were removed from the water and dried quickly with filter paper until the seed coats ceased to glisten. They were then reweighed and returned to the water as quickly as possible. Where determinations were made at close intervals of from one to three hours, the time recorded is the time the seeds were actually in the water and does not include the time during which the weighings were being made. When the determinations were made at greater intervals, however, the time is figured from the time the seeds were first immersed.

IV. EXPERIMENTAL DATA AND DISCUSSION

Asparagus seeds placed under suitable germinating conditions will germinate, whether taken directly from the mature berry, or from storage after one or two years. In fact, it is possible to get germinations of 90 per cent or more from untreated seed in one week in the laboratory, provided the temperature remains around 30° C. These facts indicate that we are not concerned here with a seed having a definite period of dormancy. If germinations of 90 per cent in one week could be obtained in the field, it would be unnecessary to hasten germination. At the time asparagus nurseries are usually planted, however, soil temperatures range from 20° C. down to about 10° C. At these temperatures asparagus seed germinates very slowly, as shown in table 1.

*Effect of Temperature upon Germination.*—The following table shows the effect of temperature upon the rate of germination of untreated asparagus seed.

TABLE 1  
RATE OF GERMINATION OF UNTREATED ASPARAGUS SEED, GERMINATED AT DIFFERENT TEMPERATURES IN THE LABORATORY

Temperature germinating chamber (° C.)	Percentage germination after:							
	3 days	4 days	5 days	6 days	8 days	10 days	12 days	17 days
10	0	0	0	0	0	0	0	0
20	0	0	2	4	11	14	.....	27
25	0	25	65	84	98	98	98	98
30	0	50	74	83	91	95	96	97
35	0	5	.....	16	31	55	67	.....
40	0	0	0	0	0	0	0	0

It will be seen from this table that the optimum temperature of germination for untreated asparagus seed is from 25° to 30° C. Germination is very slow at 20° C., and rather slow again at 35° C.

*Rate of Water Intake of Seeds Soaked at Different Temperatures.*—It was thought that determinations of the rate of water intake by asparagus seed at various temperatures might shed some light on the



cause of slow germination, so determinations were made at several temperatures between 10° and 40° C. The results are shown in table 2 and figure 1.

TABLE 2  
RATE OF WATER INTAKE BY ASPARAGUS SEEDS SOAKED AT DIFFERENT TEMPERATURES

The percentage of increase in weight is based on the original air dry weight.

Temperature soaked (° C.)	Percentage increase in weight due to soaking after:					
	4 hours	10 hours	15 hours	16 hours	22 hours	24 hours
10			11.7			14.4
18	6.1	14.4		19.6	24.0	
22	6.1	15.8		21.2	27.0	
30	15.9	27.5		33.0	36.0	
40	27.6	36.5		38.4	38.9	

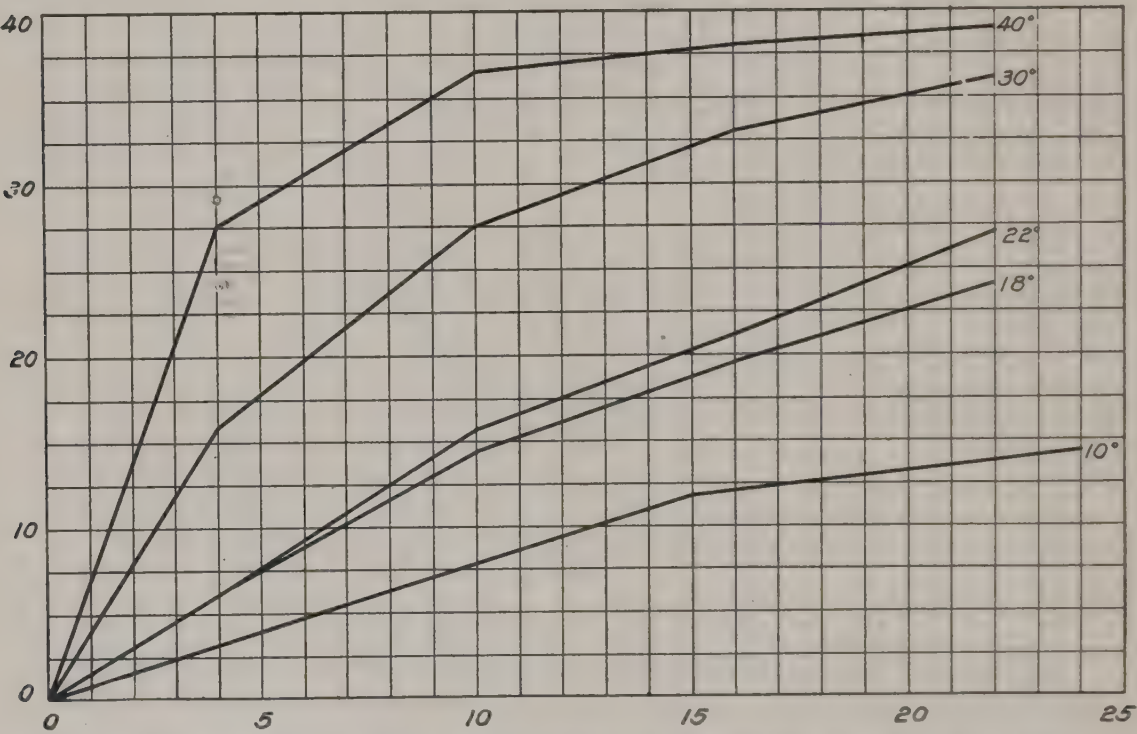


Fig. 1.—Rate of water intake by asparagus seed soaked in water at temperatures of 18° C and 30° C.

At a later date determinations of the rate of water intake were again made at temperatures of 18° and 30° C., and weighings were made for 164 hours. These results appear in table 3 and figure 2.

The results presented in table 3 and figure 2 show that seeds immersed in water at 30° C. take up very nearly their maximum amount of water about 30 hours earlier than seeds soaked at 18° C.



*Germination after Soaking in Water.*—Germination tests, in which seeds were soaked in water at different temperatures and for different lengths of time, were carried out both in the laboratory and in the field. These experiments were repeated a number of times with very uniform results.

TABLE 3  
RATE OF WATER INTAKE BY ASPARAGUS SEEDS SOAKED AT TEMPERATURES OF  
18° C. AND 30° C.

Tempera- ture soaked (° C.)	Percentage increase in weight following periods of soaking of:											
	3 hours	9 hours	24 hours	33 hours	48 hours	54 hours	69 hours	93 hours	117 hours	142 hours	153 hours	164 hours
18	5.4	13.2	28.7	34.5	40.2	41.1	42.6	43.2	43.3	.....	43.6	43.5
30	9.6	22.4	38.7	41.6	42.4	42.7	42.6	42.8	42.9	43.4	43.2	43.2

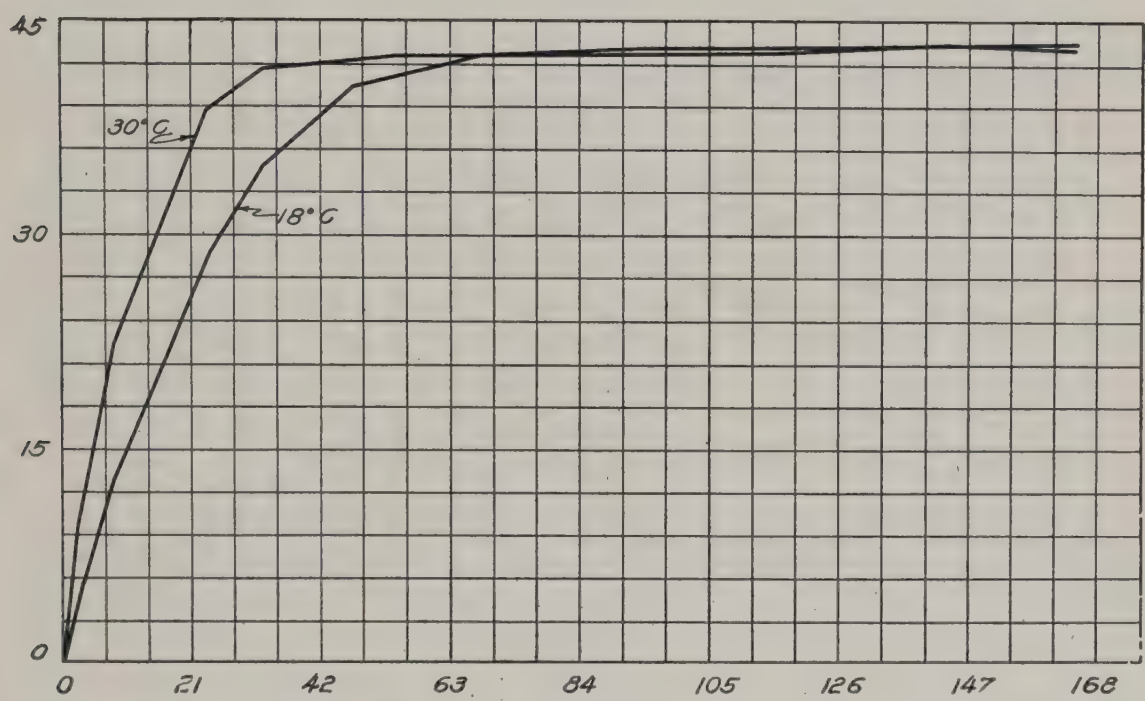


Fig. 2.—Rate of water intake by asparagus seed soaked in water at various temperatures.

*Laboratory Tests.*—In tables 4*a* and 4*b* the results are given of a series of treatments in which seeds were soaked in water for various lengths of time at temperatures ranging from 22° C. to 50° C. Daily germination percentages are shown for thirty-six sets. In Table 5 are presented data of another series similar to those of table 4*a* and 4*b*. Germination was carried out at 30° C. instead of at the somewhat lower and more variable room temperature used with the series of

TABLE 4a

EFFECT ON GERMINATION OF SOAKING ASPARAGUS SEEDS IN WATER AT VARIOUS TEMPERATURES AND FOR DIFFERENT PERIODS OF TIME. GERMINATED AT ROOM TEMPERATURE IN THE LABORATORY.

Tempera- ture soaked	Hours soaked	Per cent germination after:								
		2 days	3 days	4 days	5 days	7 days	8 days	9 days	10 days	11 days
22° C.	6	3	39	65	77	85	86	88	88	88
	14	2	13	56	66	89	90	93	93	93
	20	0	11	50	86	97	98	98	98	98
	38	12	51	75	81	88	89	90	90	92
	62	37	66	82	88	96	96	97	97	97
	86	41	64	86	88	92	93	94	94	94
	110	52	69	85	89	96	96	96	96	96
30° C.	6	0	12	54	74	93	94	95	95	95
	14	0	30	69	81	91	93	93	93	93
	20	0	50	84	92	94	96	96	96	98
	38	41	62	85	93	96	97	98	98	98
	62	45	67	78	89	94	95	95	95	95
	86	45	73	88	94	98	98	98	98	98
	110	64	86	93	95	96	97	97	97	98
38° C.	6	0	21	56	83	92	95	96	96	96
	14	3	41	69	83	89	91	92	93	93
	20	4	36	71	83	87	90	90	90	90
	38	24	74	84	92	95	95	95	95	95
	62	41	73	82	90	97	97	99	99	99
	86	39	69	82	82	94	95	95	95	95
	110	38	71	81	85	89	89	89	89	89
45° C.	6	1	13	57	71	88	90	93	93	93
	14	1	18	60	73	87	88	89	89	91
	20	0	19	63	79	91	93	94	94	94
	38	0	2	36	68	88	90	93	94	94
	62	0	0	3	12	76	85	88	90	92
	86	0	0	0	0	18	39	58	74	81
	110	0	0	0	0	0	0	0	2	3
50° C.	6	0	12	55	78	85	87	87	89	89
	14	0	14	65	87	95	95	95	95	95
	20	0	2	22	58	92	94	95	95	95
	38	0	0	3	18	60	73	82	86	86
	62	0	0	0	0	5	10	24	35	44
	86	0	0	0	0	0	0	0	0	0
	110	0	0	0	0	0	0	0	0	0
Unsoaked control.....		0	1.5	31	60	79	85	89	89	95

The control is the average of the results from two unsoaked cultures.

TABLE 4b

EFFECT ON GERMINATION OF SOAKING ASPARAGUS SEEDS IN WATER AT VARIOUS TEMPERATURES AND FOR DIFFERENT PERIODS OF TIME. GERMINATED AT ROOM TEMPERATURE IN THE LABORATORY.

Hours soaked	Tem-perature soaked (° C.)	Per cent germination after:								
		2 days	3 days	4 days	5 days	7 days	8 days	9 days	10 days	11 days
6	22	3	39	65	77	85	86	88	88	88
	30	0	12	54	74	93	94	95	95	95
	38	0	21	56	83	92	95	96	96	96
	45	1	13	57	71	88	90	93	93	93
	50	0	12	55	78	85	87	87	89	89
14	22	2	13	56	66	89	90	93	93	93
	30	0	30	69	81	91	93	93	93	93
	38	3	41	69	83	89	91	92	93	93
	45	1	18	60	73	87	88	89	89	91
	50	0	14	65	87	95	95	95	95	95
20	22	0	11	50	86	97	98	98	98	98
	30	0	50	84	92	94	96	96	96	98
	38	4	36	71	83	87	90	90	90	90
	45	0	19	63	79	91	93	94	94	94
	50	0	2	22	58	92	94	95	95	95
38	22	12	51	75	81	88	89	90	90	92
	30	41	62	85	93	96	97	98	98	98
	38	24	74	84	92	95	95	95	95	95
	45	0	2	36	68	88	90	93	94	94
	50	0	0	3	18	60	73	82	86	86
62	22	37	66	82	88	96	96	97	97	97
	30	45	67	78	89	94	95	95	95	95
	38	41	73	82	90	97	97	99	99	99
	45	0	0	3	12	76	85	88	90	92
	50	0	0	0	0	5	10	24	35	44
86	22	41	64	86	88	92	93	94	94	94
	30	45	73	88	94	98	98	98	98	98
	38	39	69	82	82	94	95	95	95	95
	45	0	0	0	0	18	39	58	74	81
	50	0	0	0	0	0	0	0	0	0
110	22	52	69	85	89	96	96	96	96	96
	30	64	86	93	95	96	97	97	97	98
	38	38	71	81	85	89	89	89	89	89
	45	0	0	0	0	0	0	0	2	3
	50	0	0	0	0	0	0	0	0	0
Unsoaked control.....		0	1.5	31	60	79	85	89	89	95

The control is the average of the results from two unsoaked cultures.



TABLE 5

EFFECT ON GERMINATION OF SOAKING ASPARAGUS SEEDS IN WATER AT VARIOUS TEMPERATURES AND FOR DIFFERENT PERIODS OF TIME. GERMINATED AT 30° C. IN THE LABORATORY.

Temperature soaked	Hours soaked	Per cent germination after:						
		2 days	3 days	4 days	5 days	6 days	7 days	8 days
20° C.	12	0	13	40	63	72	.....	81
	24	2	35	56	64	70	.....	76
	48	7	38	66	78	86	.....	87
	72	12	48	66	81	84	.....	91
	96	34	60	81	88	93	.....	96
	120	39	69	88	94	95	.....	97
	144	24	62	82	89	92	.....	95
25° C.	12	1.5	23.5	71	80.5	90.5	94	94.5
	24	13.5	55	84.5	90	93	95	95.5
	48	32	66	82	87	95.5	93.5	93.5
	96	58.5	77.5	91.5	92.5	97	98	98
	216	48	71.5	94.5	94.5	96	97	97
30° C.	12	6	23	71.5	84.5	93	95.5	96
	24	16	44	77.5	80	90	92	92
	48	44.5	68.5	86	90.5	95	95.5	96
	96	61.5	75.5	92.5	97.5	99.5	100	100
	216	33	50	81	91	91.5	96.5	96.5
35° C.	12	5	37	71	85.5	91.5	94.5	95
	24	18.5	56	79.5	86	92	97.5	97.5
	48	42	71.5	88.5	93	95	97	97.5
	96	36	66.5	85.5	93	94.5	96.5	97
	216	48.5	75	83.5	94	92	94.5	95
40° C.	12	3	35	69	86	90	91.5	95.5
	24	11	50	75	89	93	96	96
	48	22.5	60	74.5	86.5	89.5	95	96
	96	20	51.5	80	86	89.5	92.5	94.5
	216	3.5	34.5	67.5	77	85	86.5	90
50° C.	12	0	6.5	25.5	37.5	40	46.5	50
	24	0	0	16.5	32	36	39.5	40.5
	48	0	0	0	0	1.5	6.5	14.5
	96	0	0	0	0	0	0	0
	216	0	0	0	0	0	0	0
Un-soaked control.....		0.2	7.4	53.6	74.2	83.8	90.2	92.8

tables 4a and 4b. For this reason and because of minor differences in temperature-time combinations used in this series, these data can not be averaged with those of table 4a.

The date on which the seeds were placed in the germinator and not the date when soaking was begun is used as the reference point for recording germinations. Germination is impossible for most seeds while immersed in water because of an insufficient supply of oxygen and a possible excess of carbon dioxide. This was shown in an experiment with asparagus seeds in which they were soaked under several centimeters of water through which air was slowly bubbled. In one week nearly all of these seeds had germinated vigorously, whereas a control soaked in unaerated water showed no germination whatever. In fact, asparagus seeds immersed in unaerated water as long as two months have failed to germinate. The fact that germination is inhibited during a period of immersion in water has been pointed out by Kidd and West.<sup>5</sup>

Table 4a is arranged in five main divisions, each of which includes all cultures soaked at the same temperature. To make comparisons of individual cultures easier, the same results are rearranged in table 4b so that cultures soaked the same number of hours are grouped together.

The data presented in this table are the average of two identical series of cultures in all cases except cultures soaked at 20° C. and the control. The data for 20° cultures are based on single cultures while the data for the control represent the average of five unsoaked cultures.

*Field Tests—Series No. 1.*—When seeds were being soaked for the tests shown in table 4a, 200 seeds were used in each set. Half of these were germinated in the laboratory and the other half in the field. The treatment received by corresponding cultures to be tested in the laboratory and in the field was therefore exactly the same up to the time they were planted. Seed was planted at a depth of approximately three inches. This series was planted July 16, 1923, a time when soil temperatures were very high and when growth was exceedingly rapid. Asparagus nurseries are usually planted between the middle of March and the middle of May or a little later. The tests reported in table 6 were made considerably later than asparagus is ordinarily planted, but it is believed that the results are indicative of what may be expected in late planted nurseries.



TABLE 6

EFFECT OF SOAKING IN WATER AT VARIOUS TEMPERATURES FOR VARYING LENGTHS OF TIME ON GERMINATION OF ASPARAGUS SEED IN THE FIELD. PLANTED JULY 16, 1923.

Temperature soaked	Hours soaked	Per cent sprouts above ground after seeds were planted:				
		12 days	14 days	16 days	21 days	28 days
22° C.	6	1	11	15	44	45
	14	2	12	26	46	63
	20	0	3	16	39	40
	38	3	17	26	51	53
	62	8	24	31	59	54
	86	11	23	27	55	60
	110	7	14	17	39	49
30° C.	6	0	5	13	39	.....
	14	9	15	25	57	61
	20	3	11	18	52	50
	38	9	11	24	49	55
	62	14	18	38	68	79
	86	19	39	52	67	76
	110	20	30	36	57	48
38° C.	6	0	8	23	66	59
	14	4	11	29	55	54
	20	6	18	30	60	57
	38	6	16	28	50	.....
	62	13	30	35	68	53
	86	8	15	27	49	49
	110	2	24	23	65	54
45° C.	6	2	5	22	54	63
	14	8	12	16	48	54
	20	1	8	18	66	64
	38	5	16	31	54	71
	62	0	1	15	54	77
	86	0	0	0	13	35
	110	0	0	0	0	4
Unsoaked control.....	.....	0	1.75	10.5	41.75	55.75

The control is the average of four unsoaked cultures.

Table 6 shows the following facts:

1. Sixteen days after planting, all cultures shown in the table except those soaked at 45° C. for 86 or more hours have a considerably higher percentage of seedlings above ground than the unsoaked control.

2. There is no appreciable difference in final germination obtained from soaked and unsoaked seeds.



*Field Tests—Series No. 2.*—Another series, treated as indicated in table 7, was planted on March 4, 1924. The depth of planting of this series was between two and two and a half inches. The soil at the time of planting was in excellent physical condition. The seeds were placed in direct contact with finely pulverized moist soil and were immediately covered before they had any opportunity to dry. The temperature of the soil at a depth of two inches, at the time of planting and until the seeds began to come up, ranged from 10° to 23° C. There were relatively few hours when the temperature was above 20° C. during the first two weeks after the seeds were planted.

This planting which was made at a date somewhat earlier than that at which asparagus nurseries are usually planted, and than the one from which table 6 is taken, represents the opposite extremes of planting season.

In table 7 as in table 6, it is seen that many of the soaked cultures begin germination much sooner than the unsoaked cultures. It is also apparent that the final percentage germination is not appreciably changed by the treatment.

The percentages of germination on the fifty-first day after planting were, in a few cases, lower than those of the forty-seventh day. This was due to the destruction of plants by insects or other causes.

A comparison of the results shown in tables 6 and 7 with those of tables 4a and 5 shows that, in the field, there are factors operating which tend to obscure the effects of the seed treatments. For example, a culture soaked 20 hours at 22° C. (table 4a) gave 98 per cent germination eight days after it was placed on the germinator in the laboratory. This result indicates that the seeds were not harmed in any way by the treatment. In fact, this culture, in the laboratory, germinated much more quickly than the controls. Yet the duplicate lot of seeds which was soaked in the same water with these just described, produced only 40 per cent germination in the field (table 6), or a 15 per cent poorer germination than the controls.

The factors which make for lack of uniformity of germination results in the field are probably differences chiefly in depth of planting and in soil moisture. Since the appearance of primary shoots above ground was taken as the first evidence of germination, an increase of an inch in depth of planting, for example, has a marked influence on the results for at least two reasons. First, the shoot has an extra inch of growth to make before it appears above ground and,

second, the seed is in a cooler layer of soil where growth is slower. Other factors, such as insect attacks, and mechanical injuries during cultivation and counting, have some influence on the results.

*Effect of Planting in Cold Soil after Soaking in Warm Water.*—It is frequently said that soaking seed in warm water before planting in cold soil is detrimental. The results of field tests (table 7), however,

TABLE 7

EFFECT OF SOAKING IN WATER AT VARIOUS TEMPERATURES FOR VARIOUS LENGTHS OF TIME ON THE GERMINATION OF ASPARAGUS SEED IN THE FIELD. PLANTED MARCH 4, 1924.

Temperature soaked	Hours soaked	Percentage sprouts above ground after seeds were planted:							
		30 days	33 days	35 days	37 days	40 days	43 days	47 days	51 days
25° C.	12	0	0	0	0.5	4.5	38	53	67.5
	24	4	18	24	25.5	41	57	60	57.5
	48	1	3	4	11	44	64.5	73.5	71.5
	96	6	14	15.5	20	37.5	53	59	58.5
	216	9	23	27.5	30.5	39	48	52	52.5
30° C.	12	1.5	7	8.5	16.5	46	62	73	74.5
	24	0	4.5	8	10.5	20.5	30	35.5	34
	48	12	21	29	33.5	45.5	52.5	55	57
	96	16	35	40	45.5	54.5	60	62	61
	216	3.5	11	17	24	39	49	54	56
35° C.	12	0	11.5	16.5	25.5	42	50.5	54.5	56
	24	2	11.5	16	21	36	53	62.5	64.5
	48	8.5	26	31	36.5	51	63	64.5	61.5
	96	12.5	36.5	36	48.5	57.5	64	61	58
	216	6.5	33.5	43	50.5	55.5	59	64.5	63.5
40° C.	12	1	4.5	6	10.5	28	49.5	62.5	64.5
	24	2	20	27.5	40.5	46	54	59.5	58
	48	5	26	27.5	34.5	48	56.5	61.5	59
	96	0.5	21	36	43	61	66.5	67	62
	216	0	12	18	31	46.5	54	56.5	57
50° C.	12	0	0.5	3	10	27	50	70.5	75.5
	24	0	0.5	2.5	18	43.5	63	73.5	77
	48	0	0	0	0.5	5.5	14.5	29	34.5
	96	0	0	0	0	0	0	0	0
	216	0	0	0	0	0	0	0	0
Unsoaked control.....	.....	0	0.2	1.8	7.8	23.2	42.8	58.5	60.4

The control is the average of five unsoaked cultures.



indicate the contrary for asparagus seed. The seeds planted March 4, 1924 (table 7), germinated in soil, the temperature of which never exceeded 25° C., and remained below 20° C. most of the time. The highest germination obtained in the whole series was from seed soaked at 50° C. There were many cultures at all temperatures which were equal or superior to the control.

Laboratory tests (table 8) gave similar results.

TABLE 8

EFFECT OF THE TEMPERATURE OF SOAKING ON THE RATE OF GERMINATION AT LOW TEMPERATURES. SEEDS SOAKED AT 20° AND 30° C.; GERMINATED AT 20° C. IN THE LABORATORY.

Hours soaked	Temperature soaked	Percentage germination after:							
		3 days	4 days	5 days	6 days	8 days	10 days	15 days	17 days
144	20° C.	19	31	44	44	49	49	57	57
	30	19	32	41	42	47	50	50	51
120	20	9	21	35	38	40	49	49	49
	30	7	32	41	42	47	.....	50	51
96	20	10	23	34	36	38	39	41	43
	30	15	32	43	45	51	.....	.....	.....
72	20	1	10	19	32	36	39	46	46
	30	14	36	44	44	47	48	51	51
48	20	1	6	17	22	33	37	42	42
	30	9	23	31	40	43	44	46	46
24	20	0	0	5	10	17	21	26	26
	30	1	9	21	26	33	37	38	38
12	20	0	0	1	3	6	11	20	21
	30	0	0	8	11	18	21	29	30
Unsoaked control.....	.....	0	0	2	4	11	14	26	27

This table shows that asparagus seeds germinate faster even at a low temperature, if soaked before planting. Seeds soaked at 30° C. germinated more rapidly than seeds soaked at 20° C. even though both lots were germinated at 20° C. This difference in rate of germination becomes less marked with longer soaking. The final germination of asparagus seeds germinated at low temperatures is increased by soaking.



## V. SUMMARY AND CONCLUSIONS

1. In the case of *Asparagus officinalis* it is usually from two to six weeks, according to the soil and temperature conditions, before the seedlings appear above the surface. This delay may cause the grower much extra work while the plants are too small to cultivate.

2. That a period of dormancy does not occur in asparagus seed is shown by the fact that it germinates readily soon after harvesting.

3. The temperature at which untreated asparagus seed germinates most rapidly in the laboratory is between 25° and 30° C.

4. The rate of water intake by asparagus seed immersed in water at different temperatures (10° C. to 40° C.) is found to increase with the temperature of the water.

5. The maximum amount of water absorbed at any temperature is approximately 43 per cent of the original air dry weight.

6. Laboratory results show that the rate of germination of asparagus seed may be materially increased by various periods of soaking in water at different temperatures. Field results from duplicate cultures bear out these laboratory findings.

7. Asparagus seeds may be soaked for a period of nine days without reducing the final percentage of germination if the temperature does not exceed 40° C.

8. A reduction in final germination as compared with an unsoaked control may result from soaking at temperatures of more than 40° C.

9. In the laboratory tests asparagus seed soaked from two to nine days at temperatures of 20° to 38° C. germinate more quickly than seeds soaked for a shorter time under a similar temperature range. Those soaked at 25° C. to 35° C. germinated more quickly in general than those soaked at either higher or lower temperatures.

10. Laboratory and field data show that soaked seeds germinate more quickly than unsoaked seeds even though planted in cold soil.

11. For practical purposes a period of 3-5 days soaking at a temperature of 30° to 35° C. is recommended. This treatment is easily applied and the latitude of temperature and time conditions under which seed may be soaked without danger of injury makes the treatment simple and safe.

## VI. ACKNOWLEDGMENTS

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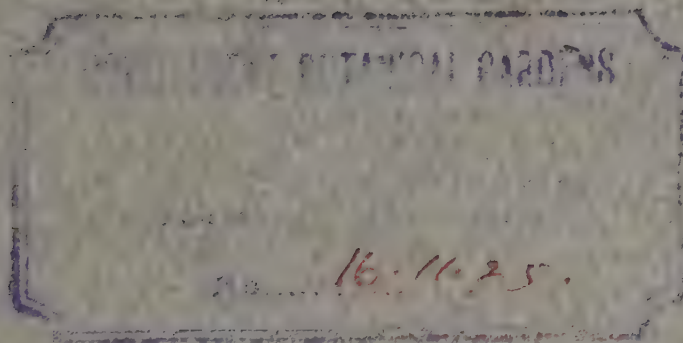
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THE RELATION OF THE SUBCUTANEOUS ADMINISTRATION OF LIVING BACTERIUM ABORTUM TO THE IMMUNITY AND CARRIER PROBLEM OF BOVINE INFECTIOUS ABORTION

BY

GEORGE H. HART AND JACOB TRAUM



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\* C. M. Haring participated in the planning of this work and C. M. Carpenter in its actual prosecution from July 1, 1922, to June 30, 1923.

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The discovery of the etiological relationship of *Bacterium abortum* to bovine infectious abortion by Bang<sup>1</sup> in 1897 naturally led this investigator to turn his attention to experiments with the organism in producing immunity to the infection. In 1906 he<sup>2</sup> reported the experiments in which he was able to produce definite protective results in sheep, goats and cattle when he injected living organisms subcutaneously<sup>3</sup> before pregnancy was established. On the other hand when the organisms had been killed by toluol before injection, little, if any, protection was afforded to the experimental animals.

Since that time McFadyean and Stockman<sup>14</sup> of England; Zwick, Zeller, Krage and Gminder<sup>23</sup>; Schermer and Ehrlich<sup>17</sup> of Germany; C. O. Jensen<sup>13</sup> of Denmark; Schroeder,<sup>15</sup> Smith and Little,<sup>18</sup> Birch and Gilman,<sup>3</sup> Hadley,<sup>8</sup> Huddleson,<sup>12</sup> Fitch and Boyd<sup>5</sup> in this country, and others have reported on the use of living and killed cultures of *Bacterium abortum* in attempts to immunize cattle against this organism. The work reported by most of these investigators consisted principally of observations on field trials under conditions in which there was no definite assurance that the treated and untreated animals picked up abortion infection and whether or not the amount of infection picked up perchance was approximately the same for both vaccinated and control animals. Other important, uncontrollable factors must have been present in these trials. In general, the results indicated that cattle injected when in a non-pregnant state with



living *Bacterium abortum* cultures produced a much higher percentage of apparently normal calvings than either the untreated animals or those which received killed organisms.

After the report made by Stockman<sup>20</sup> in 1914 of field observations on 2,150 cattle (1,279 of which were injected with live organisms, 121 with killed organisms and 750 remained as untreated controls), laboratories in many countries began the production and distribution of living abortion organisms as a vaccine for the control of bovine infectious abortion. The governments of England, Canada, South Africa, Sweden, Holland, Switzerland and others have been producing and distributing such vaccine to their stockmen. Many of the plants in the United States producing veterinary biologics are licensed by our federal Bureau of Animal Industry to manufacture and sell this product.

#### REASONS FOR THE INVESTIGATIONS

At the time we outlined our experiments some of the above-mentioned workers had not reported their results. We felt there was not sufficient information on the efficacy of live abortion organisms in the control of infectious abortion, and, rather than being a procedure to be carried out in a widespread manner in the field, it was still in the experimental stage. Even at this writing, many of the points included for investigation in our project are not satisfactorily answered by these investigators. Information based upon careful investigation regarding its efficiency in controlled experiments, the deleterious effect of its use on the vaccinated animals, the length of time the organisms remain viable in the animals, the effect on subsequent breeding, and other questions, had been so meager that general confidence in the method—and even justification for its use—was open to severe question in the minds of many investigators and livestock sanitary authorities. Frequently in this particular disease, curative and preventive measures used have been given credit for results which they did not deserve, because abortion tends to be self-limiting and may disappear without treatment. For this reason, only results from experiments which include control animals can be given very much weight.



## OUTLINE OF THE EXPERIMENTS

Our investigations were designed to furnish additional information on the important, and at that time still unsettled, question of the actual value of live abortion organisms in producing immunity. We also hoped to gain additional light on the localization, persistence, multiplication and elimination of the injected bacteria and to determine if it is necessary in the production of immunity in *Bacterium abortum* infection to have persistent multiplication and activity of the organism in the animal body or if the immunity is conferred upon an animal simply as the result of having been infected with the organism.

It was expected that the investigation would also show the extent to which the infection resulting from both the inoculation experiments to produce immunity and the ingestion experiments to produce infection would be injurious to the animals infected and also to animals associated with them.

*Source of the Cattle.*

For these purposes in the first series of experiments, 56 bovine females and 2 males were assembled. Fifteen of the females were taken from the University Dairy and five were of beef strain which had been in our possession for several years, having been originally purchased as young heifers for tuberculosis experiments but not used. All were known to be free from infection with *Bacterium abortum*. The remaining 36 females were dairy heifers purchased after a negative blood test from six herds with negative histories of abortion. The two bulls were obtained from one of the certified dairies where they had been raised. They were about fifteen months old at the time of purchase. In addition the bull at the University Dairy was used where mentioned. This animal had been with the dairy animals since August, 1917, and he, together with the other animals there, was free from *Bacterium abortum* infection as determined by extensive blood tests and milk examinations. The above animals were kept together for several months with the exceptions noted, and negative blood tests were obtained in all cases before the experiments began. They were divided into four groups as follows:

Group I, consisting of 20 animals, was the group used to determine the efficiency of the injection of live abortion organisms in preventing abortion.

Group II, consisting of 15 animals, constituted the controls, divided into two sub-groups:

A—Ten head to actually receive infectious material to produce abortion;

B—Five head left as association animals.

Group III, consisting of 10 animals, received vaccine but no further treatment in order to ascertain how long *Bacterium abortum* would remain in their bodies as a result of a single exposure by subcutaneous injection; also, to ascertain if the organism would be given off in the colostrum and placentae of these animals at the first subsequent parturition. The effect of the vaccination could be studied in these animals as well as in those of Group I.

Group IV, consisting of 11 animals, divided into two sub-groups:

A—Five head bred, without previous treatment, by the bulls after they had served the vaccinated animals in Groups I and III.

B—Six head left open so that in case opportunity offered they could be bred by the bulls shortly after breeding an aborting cow.

This group was intended to constitute a check on the possibility of exposed bulls transmitting the infection, through the medium of copulation, to non-infected females.

Table 1 gives the lists of these groups with the agglutination tests throughout the period covered by this report.

### *Distribution of the Cattle.*

When the animals were divided into groups, they were placed in separate fields (fig. 1). The pastures used for the experiments comprise the north side of a cañon. The land is sloping and hilly and the drainage is in one direction from the hillside into the creek at the bottom of the cañon (from the upper to the lower part of fig. 1).

Drainage from the far-east pasture can, therefore, run through the east pasture and from the connecting pasture into the area occupied by the buildings and the road pasture. The remainder of the land drains directly into the creek. These facts were kept in mind in placing the animals so that infection from one group to another would not take place through the medium of drainage.



### *Preparation of the Vaccine.*

Four strains of the abortion organism were used in making the suspension. Two of these, A and 80, were old bovine laboratory strains which grew very rapidly and heavily on culture media. The third was a strain obtained from live abortion germ vaccine sold by a commercial firm in this country, and the fourth, 101, was a strain isolated (October 26, 1921) in this laboratory from an aborted bovine fetus. The cultures were grown on glucose glycerine bouillon and glucose glycerine agar, the growth on the latter being washed off with

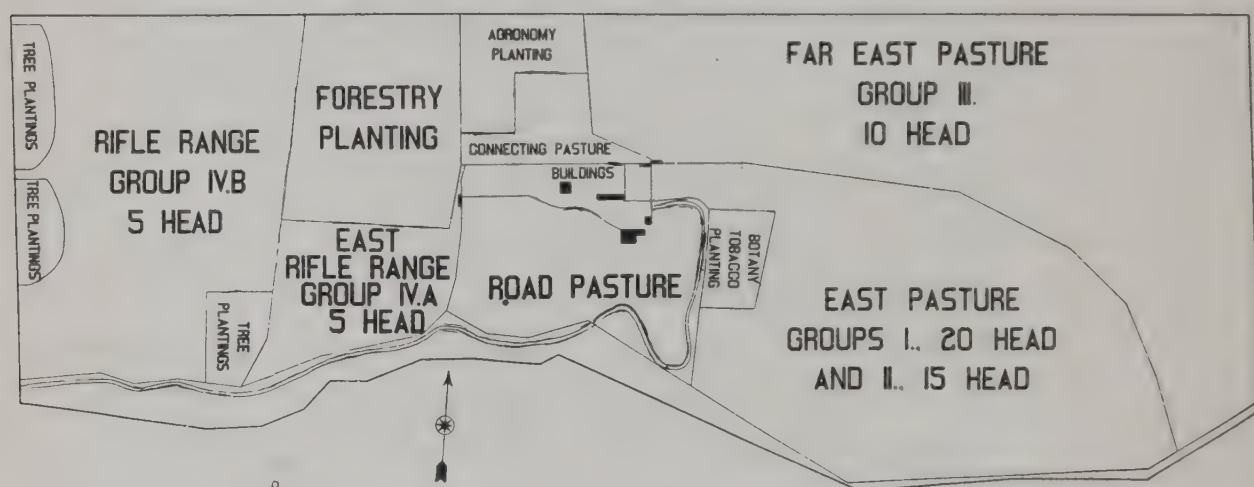


Fig. 1. Plot, plan of buildings and pastures in Strawberry Cañon, University of California, Berkeley, occupied by experimental cattle in abortion investigations.

saline solution and used to enrich the bouillon cultures. Subcultures were made and smears stained from each of the flasks which showed them to be pure cultures of the organism. All of the strains were known to be pathogenic for guinea pigs. The suspension of the organisms was tested with a silica comparator standard using Pear's precipitated fullers' earth. The technique of this preparation is given in the turbidity standard of the Standard Methods of Water Analysis by the American Public Health Association as used by Butterfield and Neill<sup>4</sup> in the Hygienic Laboratory in their work on various strains of meningococci.

In this work we desired to use organisms of known pathogenicity in a dose as high as that generally used in order that failure to produce protection could not be ascribed to lack of virulence or number of organisms. The silica comparator standard used had been prepared for meningococci. When the suspension of *Bacterium abortum* equalled the four billion per mil meningococci standard, it appeared as opaque as any of the samples of commercial abortion vaccine in



our possession and this was therefore the concentration used. When this was later tested with the Gates<sup>7</sup> opacimeter, it showed a reading of .9 cm. When the organisms in such a suspension were diluted and counted by the plate culture method, it yielded an average of eleven billion organisms per mil.

#### *Preparation of Infectious Material.*

Eight gallons of milk, from cows under observation by Hayes and Barger at the University Farm, Davis, were secured for use in this experiment. The milk from these cows was known to contain *Bacterium abortum*. To test this particular milk, 800 mls were centrifuged and the sediment inoculated intra-abdominally into guinea pigs 2334 and 2335. No. 2334 died soon after inoculation and was not autopsied. No. 2335 was killed at the end of eight weeks and found to have extensive lesions of *Bacterium abortum* infection. Its blood gave a positive agglutination test.

The following material from bovine fetuses, which had been received at the laboratory and found to contain *Bacterium abortum*, was mixed in salt solution to a volume of 1 gallon:

Fetus Number	Lungs	Stomach Contents	Intestinal Contents	On Ice Since
32	×	×	×	May 13
33	×	----	----	May 30
35	×	×	----	May 25
37	×	×	----	June 1
38	×	×	----	June 12
40	×	×	----	June 15

A bottle of 1-gallon capacity was used to hold 1250 mls of glycerine glucose broth culture of *Bacterium abortum*, strain 4, also the surface growth of strain 80 on 13 bottles of glycerine glucose agar washed off with salt solution. Strain 4 was isolated from the abscess of cow 4 following vaccination—80 was an old laboratory bovine culture and one of the strains used in the vaccine.

A third gallon bottle was used to hold 2000 mls of broth culture of strain 118 isolated in this laboratory March 10, 1922, from fetus 18.

A fourth gallon bottle was used to hold surface growth on fetus media agar, washed off with salt solution, of strains of *Bacterium abortum* recently isolated from fetuses 10, 20, 35, 37, 38, and 40.

A fifth gallon bottle was used to hold strains of *Bacterium abortum* on solid and liquid media isolated from guinea pigs inoculated with tissues of infected fetuses 3, 10, 11, and 18.

There were thus available 8 gallons of naturally infected milk, 1 gallon of infected fetus tissues in salt solution, and 4 gallon bottles containing cultures of *Bacterium abortum* and each filled to a gallon volume with water at the time of the infection, 6.30 to 9.30 p.m., June 26, 1922.

#### *Method of Preparing Colostrum and Placentae for Guinea Pig Inoculations from the Animals of All Groups.*

The colostrum for injecting the guinea pigs, as shown in table 2, was obtained in sterile pint jars immediately after calving. From 300 to 500 mils were taken in each case, some being collected from each of the four teats. This was brought to the laboratory and centrifuged in 100-mil centrifuge tubes for twenty minutes. Some of the fat from the surface and the sediment from the bottom of one or two tubes were mixed together and 1 to 2 mils injected intra-abdominally into each guinea pig.

The entire placenta, or as much of it as could be collected in each case, was placed in a sterile 1-gallon covered can and brought to the laboratory. In case it was soiled with manure or bedding, it was washed in tap water. It was then spread out on a tray and a careful examination made for any evidence of necrotic, hemorrhagic or other abnormal areas. Material for guinea pig injection was always taken from the most suspicious looking areas. This was ground in a mortar with sterile salt solution and injected intra-abdominally into guinea pigs.

Stained smears were also examined microscopically in each case.

#### EXPERIMENTAL DATA ON ANIMALS OF GROUP I

On February 7, 1922, the animals of this group were placed in the road pasture with those of Group III (fig. 1), and injected with the vaccine. Each animal was given subcutaneously, at one point on the left side of the neck, 20 mils of the material. The injected area was previously washed with a 3 per cent compound cresol solution as would be done in routine field practice.



The day after the injection a cold rain-storm began and continued four days. Practically all of the animals had a more or less marked reaction, probably exaggerated by the bad weather conditions. They stood humped up in the pasture and ate very little. Alfalfa hay was being fed to them at the time. By February 11, they were all eating normally and on the following day the weather had cleared and they appeared to have recovered from the effects of the vaccination. However, local swellings were present on all of the animals at the point of injection. In the majority of cases these increased in size for several weeks and, in a number of the cattle involved the prescapular lymph gland on the side injected. On March 10, 31 days after vaccination, the following conditions were found on examination of the injected areas:

- No. 4. Large abscess.
- No. 25. Large, soft abscess, 4" by 6" by 3". Opened by incision and material taken for culture.
- No. 403. Normal.
- No. 404. Enlarged gland.
- No. 405. Normal.
- No. 407. Normal.
- No. 408. Gland enlarged and hard.
- No. 410. Enlarged gland.
- No. 414. Soft abscess.
- No. 415. Small abscess.
- No. 418. Enlarged gland.
- No. 421. Abscess had opened naturally.
- No. 424. Enlarged gland.
- No. 426. Abscess had opened naturally.
- No. 428. Large abscess, 6" by 4". Opened.
- No. 433. Slight swelling.
- No. 434. Abscess had opened naturally.
- No. 2182. Large abscess.
- No. 2305. Gland slightly enlarged.
- No. 2314. Abscess had opened naturally.

These animals at that time were not in such good condition as the 13 controls in Group II. The pus from the abscesses was identical in all cases, being thick yellowish-white in appearance. This condition probably would have been avoided to a considerable extent had the suspension of the organisms been further diluted and the injection made in several areas instead of placing the entire 20 mils at one point. Pus was collected from the abscesses on cows 4 and 25. Inoculations made from this material developed pure cultures of



*Bacterium abortum* from cow 4 and *Bacterium abortum* with some contamination from cow 25.

On February 21, 1922, fourteen days after vaccination, blood samples were taken from these animals and all gave a positive reaction to the agglutination test (see table 1).

The breeding of these animals was begun April 10, 1922, sixty-two days after the vaccination, when the bulls were removed from Group II and kept corralled so that breeding dates could be secured.

The animals in this group, although they had apparently entirely recovered from the effect of the vaccination, came in heat slowly. The following breeding took place:

- Bull 412 bred on April 18, 1922, to no. 433.
- Bull 412 bred on April 19, 1922, to no. 434.
- Bull 412 bred on April 20, 1922, to no. 2314.
- Bull 412 bred on April 20, 1922, to no. 403.
- Bull 412 bred on April 22, 1922, to no. 408.
- Bull 412 bred on April 26, 1922, to no. 421.
- Bull 412 bred on April 27, 1922, to no. 428.
- Bull 412 bred on April 27, 1922, to no. 2182.
- Bull 412 bred on May 3, 1922, to no. 410.
- Bull 412 bred on May 11, 1922, to no. 424.
- Bull 411 bred on April 21, 1922, to no. 25.
- Bull 411 bred on April 27, 1922, to no. 407.
- Bull 411 bred on April 29, 1922, to no. 414.
- Bull 411 bred on April 29, 1922, to no. 434.
- Bull 411 bred on May 1, 1922, to no. 418.
- Bull 411 bred on May 19, 1922, to no. 405.

When the breeding of this group was begun, these animals, with those of Group III, were placed in the east pasture where there was good green feed. After breeding, each animal was removed to the road pasture.

On May 12, 1922, on account of the animals breeding slowly and time being an important factor owing to the control animals (Group II) being pregnant, a rectal examination was made of the unbred animals. The ovaries were massaged and the corpora lutea were expressed from nos. 404, 405, and 426.

On June 24, 1922, the 20 animals of Group I were separated from the ten of Group III and kept in the road pasture. They had all been bred, but on this date they were examined for pregnancy. Some of them had been too recently bred for this to be of any value. The result of the examination is shown below:

No. 4.	Bred April 19.	Pregnant.
No. 25.	Bred April 21.	Pregnant.
No. 403.	Bred April 20 and June 3.	?
No. 404.	Bred June 20.	?
No. 405.	Bred May 19 and June 19.	?
No. 407.	Bred April 27.	Pregnant.
No. 408.	Bred April 22.	Pregnant.
No. 410.	Bred May 3.	Pregnant.
No. 414.	Bred April 29.	Pregnant.
No. 415.	Bred June 10.	?
No. 418.	Bred May 1.	Pregnant.
No. 421.	Bred April 26 and June 3.	?
No. 424.	Bred May 11.	?
No. 426.	Bred June 24.	?
No. 428.	Bred April 27.	Pregnant.
No. 433.	Bred April 18.	Pregnant.
No. 434.	Bred April 29.	Pregnant.
No. 2182.	Bred April 27.	Pregnant.
No. 2305.	Bred May 25.	?
No. 2314.	Bred April 20.	Pregnant.

A rectal examination only was made as most of them were heifers and to get the hand into the vagina was difficult or impossible. Also we<sup>21</sup> hold the opinion that under certain unrecognized conditions, bimanual examination may be the cause of abortion in a small percentage of cases.

In this group, cows 405, 415 and 428 were later found not to have conceived. No. 428 on June 24 was thought to be pregnant, having been bred on April 27, fifty-eight days prior to the examination. It is believed that this diagnosis of pregnancy was in error rather than that she aborted, since she was being daily observed with other animals in the group and was seen to be in heat on August 5.

This group of animals was kept corralled from June 24 to 26 with the animals of Group II-A. This was done to control their water supply in the hope that they would drink from the watering-trough the infectious material to be given them on the latter date.

The administration of the infectious material was delayed until late in the day in order that it would not be exposed to strong light during the process. An effort was made to mix the material in the drinking water. On account of discoloration of the water by the milk and a slight odor from the fetus material, the animals would not drink, although they had had little water for the previous forty-eight hours. They were then placed in the chute and drenched.

Five drenching batches were made by taking 500 mls from each of the five 1-gallon bottles and 500 mls of milk, making a total of 3000 mls, of which mixture each cow was drenched with approximately 1 pint. The remainder of the infectious mixture was placed in the watering-trough, baled alfalfa hay was opened and the flakes were soaked in the trough until the solution was absorbed. It was then spread around the corral for the animals to eat. They had not been previously fed on that day and no difficulty was experienced in getting them to eat the hay. The next morning the animals were turned into the east pasture. The watering-trough in the corral was disinfected and no further infection was given to the cattle.

*History of First Pregnancy of Animals of Group I, Vaccinated and Infected.*

The parturition history of the 20 animals in Group I, which were kept with the animals in Group II after infection and subjected to the same conditions except that the former had been vaccinated with live abortion organisms seventy days or longer prior to breeding, is given in table 2.

From an examination of the data it will be seen that every one of the 17 animals in this group that became pregnant carried their calves to term except no. 407, which was accidentally killed in the last month of gestation. Pregnancy was progressing normally and no evidence of abortion infection could be found in her tissues, the results of the examination of which will be discussed later.

The following examination and notes were made of the fetus:

Fetus of cow 407: Removed from uterus after death of dam caused by broken neck; black and white female; 8 months gestation.

Externally: Normal.

Internally: Tissues normal.

Heart: Few petechial hemorrhages on myocardium of ventricles.

Stomachs: Distended with a viscid, faintly-clouded fluid, which showed no evidence of being stained with meconium.

Rectum: Meconium made up of firm, mucous-coated pellets, greenish in color.

Cultures:

Heart's blood,	negative
Lung,	negative
Liver,	negative
Spleen,	negative
Stomach contents,	negative
Small intestine,	negative
Meconium rectum,	negative



TABLE 2.—BREEDING AND CALVING DATES OF EXPERIMENTAL ANIMALS WITH RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF BACTERIUM ABORTUM

FIRST PREGNANCY

No. of Animal	Breeding date	Calving date	Guinea pigs injected Placenta	Guinea pigs injected Colostrum	Guinea pigs killed	Post mortem findings Guinea pigs	Cultures from spleens of Guinea pigs	Blood reaction of Guinea pigs	Placenta
GROUP I									
4	Apr. 19, 1922	Jan. 23, 1923	2848-49	2846-47	Mar. 14, 1923	2846 + Others —	2846 + Others —	2846 + Others —	Expelled normally
25	Apr. 21, 1922	Feb. 7, 1923	2923-24	2921-22	Mar. 26, 1923	—	—	—	Expelled normally
403	Apr. 20, 1922; June 3, 1922	Mar. 4, 1923	3015-16	3013-14	Apr. 15, 1923	—	—	—	Expelled normally
404	June 20, 1922	Apr. 1, 1923	3123-24	3125-27	May 16, 1923	—	—	—	Expelled normally
405	May 19 and June 19, 1922.								
	Did not conceive.								
407	Apr. 27, 1922	Died	2782	.....	Feb. 13, 1923	—	—	—	
408	Apr. 22, 1922	Dec. 28, 1922	2864-65	2862-63	Mar. 15, 1923	2863 + Others —	2863 + Others —	2863 + Others —	Expelled normally
410	May 3, 1922	Feb. 2, 1923	2889-90	2891-92	Mar. 16, 1923	—	—	—	Expelled normally
414	Apr. 29, 1922	Feb. 9, 1923	2934-35	2932-33	Mar. 26, 1923	—	—	—	Expelled normally
415	June 10, 1922. Did not conceive.								
418	May 1, 1922	Feb. 2, 1923	2893-94	2895-96	Mar. 16, 1923	—	—	—	Expelled attached to calf
421	Apr. 26, 1922; June 3, 1922	Mar. 6, 1923	3025-26	3023-24	Apr. 25, 1923	3023-24 + Others —	3023-24 + Others —	3023 + Others —	Expelled normally
424	May 11, 1922	Feb. 7, 1923	2927-28	2925-26	Mar. 26, 1923	—	—	—	Expelled normally
426	June 24, 1922	Mar. 24, 1923	3066-67	3080-81	3066 died Mar. 26, 1923 Others killed May 4, 1923	3080-81 + Others —	3080-81 + Others —	3080-81 + Others —	Expelled normally
428	Apr. 27, 1922. Did not conceive.								
433	Apr. 18, 1922	Jan. 20, 1923	2834-35	2836-37	Mar. 1, 1923	—	—	—	Calved 9 A.M. Placenta not passed at 11 A.M. Removed and some cotyledons adherent.
434	Apr. 20, 1922	Feb. 2, 1923	2904-05	2902-03	Mar. 19, 1923	—	—	—	Expelled normally
2182	Apr. 27, 1922	Jan. 23, 1923	2860-61	2858-59	Mar. 15, 1923	—	—	—	Expelled normally
2305	May 25, 1922	Feb. 27, 1923	2995-97 2999-3000	2985- 2995-3000	2995-97-99 died. Others killed Apr. 15, 1923 Mar. 1, 1923	—	—	—	Retained
2314	Apr. 20, 1922	Jan. 20, 1923	2838-39	2840-41	Mar. 1, 1923	—	—	—	Expelled normally

TABLE 2.—BREEDING AND CALVING DATES OF EXPERIMENTAL ANIMALS WITH RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF BACTERIUM ABORTUM—(Continued)

FIRST PREGNANCY

No. of Animal	Breeding date	Calving date	Guinea pigs injected Placenta	Guinea pigs injected Colostrum	Guinea pigs killed	Post mortem findings Guinea pigs	Cultures from spleens of Guinea pigs	Blood reaction of Guinea pigs	Placenta
GROUP II									
20.....	Between Feb. 7 and Apr. 10, 1922.	Aborted Sept. 10, 1922	2532	2538	Nov. 2, 1922	+	+	+	Retained
26.....	do.....	Dec. 3, 1922	2731	2732	Jan. 19, 1923	—	—	—	Expelled normally
401.....	Pregnant when purchased.....	July 23, 1922	2357-65	2378	Sept. 25-27, 1922.	2365 — 2357-78+	2357-78+ Other —	2357-78+ Other —	Retained
416.....	Between Feb. 7 and Apr. 10, 1922.	Aborted Sept. 7, 1922	2531	2539	Nov. 2, 1922	+	+	+	Expelled normally
429.....	do.....	do.....	2528	2537	Nov. 2, 1922	+	+	+	Retained
431.....	do.....	Aborted Sept. 20, 1922	2574	2577	Nov. 7, 1922	+	+	+	Retained
2060.....	Sept. 26, 1921.....	Sept. 6, 1922	.....	2344	Sept. 12, 1922	—	—	—	Expelled normally
2180.....	Between Feb. 7 and Apr. 10, 1922.	Aborted Sept. 2, 1922	2504	2507	Nov. 2, 1922	+	+	+	Retained
2181.....	do.....	Aborted Aug. 21, 1922	2449	.....	Oct. 23, 1922	+	+	+	Retained
2312.....	do.....	Dec. 3, 1922	2725	2724	Jan. 19, 1923	—	—	—	Expelled normally
183.....	May 3, 1922.....	Feb. 16, 1923	2965-66	2951-52	Mar. 30, 1923	2965-66 + Others —	2965-66 + Others —	2965-66 + Others —	Expelled normally
430.....	July 22, 1922.....	Apr. 27, 1923	3198-99	3196-97	3198-99 died 3196-97 killed	3196 + 3197 —	3196 + 3197 —	3196 + 3197 —	Expelled normally
436.....	Pregnant when purchased	Apr. 3, 1923	3128 29	3130-31	June 29, 1923	—	—	—	Expelled normally
2298.....	Apr. 24, 1922.....	Feb. 3, 1923	2908-09	2906-07	May 16, 1923 Mar. 19, 1923	2906 + Others —	2906 + Others —	2906 + Others —	Retained
2317.....	June 11, 1922.....	Mar. 16, 1923	3038-39	3040-41	3038 died Apr. 25, 1923	3040 41 + Other —	3040 41 + Other —	3040 41 + Other —	Expelled normally

TABLE 2.—BREEDING AND CALVING DATES OF EXPERIMENTAL ANIMALS WITH RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF BACTERIUM ABORTUM—(Continued)

FIRST PREGNANCY

No. of Animal	Breeding date	Calving date	Guinea pigs injected Placenta	Guinea pigs injected Colostrum	Guinea pigs killed	Post mortem findings Guinea pigs	Cultures from spleens of Guinea pigs	Blood reaction of Guinea pigs	Placenta
GROUP III									
402.....	Did not get pregnant.								
406.....	Did not get pregnant.								
413.....	Apr. 10, 1922 .....	Jan. 17, 1923	2826-27	2824-25	2825 died Others killed Mar. 1, 1923	—	None made	—	Expelled normally
419.....	Did not get pregnant.								
2297.....	After June 24, 1922 .....	Apr. 7, 1923	3140-41	3138-43	3140-41 died 3138-43 killed May 24, 1923	—	—	—	Expelled normally
2313.....	Did not get pregnant.								
2315.....	Did not get pregnant.								
2318.....	After June 24, 1922 .....	Apr. 6, 1923	3134-35	3132-33	May 24, 1923	—	—	—	Expelled normally
2319.....	Did not get pregnant.								
2321.....	After June 24, 1922 .....	May 14, 1923	3223-24	3221-22	June 29, 1923	—	—	—	Expelled normally
GROUP IV									
435.....									
437.....									
A { 438.....									
439.....									
445.....									
440.....									
441.....									
B { 442.....									
443.....									
444.....									
446.....									

Not in experiment the first year.



TABLE 2.—BREEDING AND CALVING DATES OF EXPERIMENTAL ANIMALS WITH RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF BACTERIUM ABORTUM—(Continued)

## SECOND PREGNANCY

No. of Animal	Breeding date	Calving date	No. of Calf	Guinea pigs injected Placenta	Guinea pigs injected Colostrum	Guinea pigs killed	Blood Reaction of Guinea pigs	Post mortem Guinea pigs	Cultures from spleens of Guinea pigs	Placenta
GROUP I										
4	May 14, 1923	Feb. 22, 1924	A. E. 52	3673-74	3675-76	3674 died Others killed Apr. 4, 1924 Apr. 24, 1924	3676 Susp. Others —	3676 Susp. Others —	3675-76 + Others —	Manually removed—not adherent. Metritis followed. Expelled normally
25	May 14 and 26, 1923	Mar. 9, 1924	A. E. 61	3724-35	3722-23		—	—	—	
403	Apr. 25, 1924	Second	pregnancy not yet terminated.				breeding.			
404	Aug. 2, 1923	May 14, 1924	A. E. 72	3846-47	3848-49	July 2, 1924	—	—	—	Expelled normally
405	May 14, 1923	Feb. 20, 1924	A. E. 50	3652-53	3650-51	Apr. 3, 1924	—	—	—	Expelled normally
407	Died during first pregnancy.									
408	May 12, 1923	Feb. 23, 1924	A. E. 53	3679-80	3677-78	3680 died Mar. 21, 1924 Others killed Apr. 8, 1924	3677 + Others —	3677 + Others —	3677 + Others —	Expelled normally
410		Second	pregnancy not yet terminated.							
414		June 22, 1924	A. E. 74	3908	3907	Aug. 13, 1924	.....	.....	.....	Expelled normally
415		Mar. 4, 1924	A. E. 60	3703-10	3707-08	Apr. 18, 1924	—	—	—	Expelled normally and eaten
418	Apr. 25, 1924	Second	pregnancy not yet terminated.				breeding.			
421		Killed at end of first pregnancy.								
424		Killed at end of first pregnancy.								
426	June 12, 1923	Mar. 19, 1924	A. E. 64	3750-51	3748-49	May 5, 1924	—	—	—	Expelled normally
428		Only animal in experiment which never became pregnant.								
433	May 3, 1923	Feb. 8, 1924	A. E. 45	3584-85	3586-87	3584 died Feb. 10, 1924 Others killed Mar. 21, 1924	—	—	—	Expelled normally
434	July 23, 1923	May 2, 1924	A. E. 70	3818 19	3816 17	Mar. 24, 1924	—	—	—	Expelled normally
2182	May 2, 1923	Feb. 11, 1924	A. E. 48	3530-91	3588 89	Mar. 24, 1924	—	—	—	Manually removed. In part adherent.
2305	Dec. 14, 1923	Oct. 4, 1924	A. E. 76	4071 72	4069 70 73	4071 72 70 died. Others killed Nov. 18, 1924 Mar. 21, 1924	—	—	—	Expelled normally
2314	May 3, 1923	Feb. 8, 1924	A. E. 47	3580-81	3582-83		—	—	—	Manually removed. Adherent.

TABLE 2.—BREEDING AND CALVING DATES OF EXPERIMENTAL ANIMALS WITH RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF BACTERIUM ABORTUM—(Continued)

SECOND PREGNANCY

No. of Animal	Breeding date	Calving date	No. of Calf	Guinea pigs injected Placenta	Guinea pigs injected Colostrum	Guinea pigs killed	Blood Reaction of Guinea pigs	Post mortem Guinea pigs	Cultures from spleens of Guinea pigs	Placenta
GROUP II										
20.....	Feb. 12, 1923	Nov. 20, 1923	A. E. 42	3483-84	3481-82	Jan. 8, 1924	3481-82 + Others —	3481-82 + Others —	3481-82 + Others —	Expelled normally
26.....	May 23, 1923	Mar. 4, 1924	A. E. 59	3703-04	3705-06	Apr. 18, 1924	—	—	—	Expelled normally
401.....	May 19, 1923	Feb. 28, 1924	A. E. 55	3685-86	3687-88	Apr. 11, 1924	3687-88 + Others —	3687-88 + Others —	3687-88 + Others —	Expelled normally
416.....	Feb. —, 1923	Nov. 13, 1923	A. E. 41	3447-48	3445-46	Jan. 7, 1924	+	+	+	Manually removed but not adherent
439.....	Apr. 7, 1923	Jan. 3, 1924	A. E. 43	3539-40	3537-38	3538 died Jan. 17, 1924 Others killed Feb. 14, 1924 Dec. 11, 1923	3537 + Others —	3537 + Others —	3537 + Others —	Expelled normally
431.....	May 27, 1923	Oct. 22, 1923	A. E. 40	3428-29	.....	.....	—	—	None made	
2060.....	.....	.....	to get pregnant second time. Killed.	.....	.....	.....	.....	.....	.....	
2180.....	May 14 and July 22, 1923	Apr. 27, 1924	A. E. 69	3814-15	3812-13	June 14, 1924	3812-13 + 3814-15 —	3812-13 + 3814-15 —	3812-13 + 4814-15 —	Expelled normally
2181.....	May 13, 1923	Feb. 19, 1924	A. E. 49	3644-45	3646-47	Apr. 3, 1924	3646-47 + Others —	3646-47 + Others —	3646-47 + Others —	Expelled normally
2312.....	Apr. 25, 1923	Jan. 31, 1924	A. E. 44	3559-60	3557-58	Mar. 13, 1924	—	—	—	Manually removed but not adherent
183.....	May 13, 1923	Feb. 29, 1924	A. E. 56	3689-90	3691-92	Apr. 11, 1294	—	—	—	Manually removed but not adherent
430.....	Aug. 6, 1923	May 17, 1924	A. E. 73	3853-54	3855-56	July 2, 1924	—	—	—	Expelled normally
436.....	Dec. 25, 1923	Oct. 6, 1924	A. E. 77	4076-77	4074-75	Nov. 18, 1924	—	—	—	Expelled normally
2298.....	May 14, 1923	Feb. 25, 1924	A. E. 54	3683-84	3681-82	Apr. 8, 1924	3681-82 + Others —	3681-82 + Others —	3681-82 + Others —	Expelled normally
2317.....	May 9, 1923	Feb. 20, 1924	A. E. 51	3671-72	3669-70	Apr. 4, 1924	—	—	—	Expelled normally



TABLE 2. BREEDING AND CALVING DATES OF EXPERIMENTAL ANIMALS WITH RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF BACTERIUM ABORTUM (Concluded)  
SECOND PREGNANCY

No. of Animal	Breeding date	Calving date	No. of Calf	Guinea pigs injected Placenta	Guinea pigs injected Colostrum	Guinea pigs killed	Blood Reaction of Guinea pigs	Post-mortem Guinea pigs	Cultures from spleens of Guinea pigs	Placenta
<b>Group III</b>										
402	June 1, 1923	Mar. 22, 1924	A. F. 66	3759-60	3757-58	May 5, 1924	—	—	—	Expelled normally
406	June 13 and Sept. 20, 1923	June 26, 1924	A. F. 75	Placenta lost	3909-10	Aug. 13, 1924	—	—	—	Expelled normally
413	June 10 and July 2, 1923	Killed at end of first pregnancy.								
415	June 10 and July 2, 1923	Apr. 5, 1924	A. F. 67	3765-66	3767-68	May 19, 1924	—	—	—	Expelled normally
2257	May 9, 1923	Killed at end of first pregnancy.								
2311	May 9, 1923	Feb. 8, 1924	A. F. 46	3576-77	3578-79	3576-77 Mar. 18, 1924	—	—	—	Manually removed but not adherent
2315	June 2, 1923	Mar. 12, 1924	A. F. 62	3730-31	3728-29	Mar. 21, 1924	—	—	—	Expelled normally
2312	June 2, 1923	Mar. 13, 1924	A. F. 63	3732-33	3736-37	Apr. 24, 1924	—	—	—	Expelled normally
2317	June 29, 1923	Apr. 12, 1924	A. F. 62	3703-70	3771-72	May 30, 1924	—	—	—	Expelled normally
2321	June 2, 1923	Mar. 19, 1924	A. F. 65	3745-47	3744-45	May 5, 1924	—	—	—	Expelled normally
<b>Group IV</b>										
433	Dec. 1, 1922	Oct. 6, 1923	A. F. 38	None injected	None injected		—	—	—	Expelled normally. Lost
437	Dec. 1, 1922	Died	Ruptured uterus	3381-82	.....	Died	—	—	—	
432	Mar. 24 and May 21, 1923	Sept. 26, 1923 Mar. 3, 1924	A. F. 54	3761-62	3434-3760	Sept. 28, 1923 Apr. 12, 1924	—	—	—	Manually removed but not adherent
439	Apr. 1, 1923	May 10, 1924	A. F. 71	3273-30	3227-28	June 20, 1924	—	—	—	Expelled normally
445	May 27, 1923	Mar. 2, 1924	A. F. 57	3635-36	3537-38	Apr. 12, 1924	—	—	—	Expelled normally
440	.....	Not used in experiment.	in experiment.							
441	.....	Not used in experiment.	in experiment.							
442	Jan. 2, 1923	Oct. 9, 1923	A. F. 39	None injected	None injected		—	—	—	Expelled normally and partly eaten
443	.....	Not used in experiment.	in experiment.							
444	Apr. 7, 1923	Jan. 11, 1924		3545-46	3543-44	3545-46, died Jan. 13, 1924 3543-44 Children killed Feb. 28, 1924	—	—	—	Expelled normally and partly eaten
446	.....	Not used in experiment.	in experiment.							



Blood serum of the calf was negative to the agglutination test.

Smears made from stomach contents were negative.

Guinea pig 2143: Inoculated with stomach contents; killed February 12, 1923; negative.

Guinea pig 2144: Inoculated with extract of the lung, liver and spleen; killed February 13, 1923; negative.

All of the animals in this group passed their placentae normally except nos. 422 and 2305. The former calved at 9 A.M. and since the placenta was desired for examination, it was manually removed at 11 A.M. Some of the cotyledons in the apex of the pregnant horn were markedly adherent. This afterbirth, however, might have passed normally had more time been given.

No. 2305 calved at 11 A.M., with assistance from the attendant, after having been in labor since 7 A.M. This was a small heifer and the calf was large and expelled dead. Postmortem examination showed the lungs had not been inflated. While it was an anterior presentation, death may have occurred during parturition or may have resulted from inflammation of the placenta which was present. The following day at 11 A.M. part of the placenta was protruding from the vagina and was torn off and placed in a sterile can by the attendant. At 2 P.M. a quantity sufficient to nearly reach the floor had been ejected. On removing this and making a manual examination, shreds of tissue were found to be still adherent to the maternal cotyledons and there was considerable discharge from the uterus. This was, therefore, a definite case of retained placenta. Three days later the heifer was again examined and shreds of the placenta were still found to be attached to the uterus.

The first two guinea pigs inoculated with placental material died in forty-eight hours. Two others were then inoculated with uterine exudate. One of these also died in forty-eight hours but the other lived. This latter was finally killed at the end of six weeks and was negative for *Bacterium abortum*.

The following examination notes were made of this calf:

Expelled dead from dam 2305, 11 A.M., February 27, 1923; apparently mature and well developed; black and white; female.

Externally: Normal.

Internally: Tissues appeared normal.

Heart: Base of ventricle heavily spotted with petechial hemorrhages.

Lungs: Normal, not inflated.

Liver, Spleen and Kidney: Normal.

Stomachs: Filled with a clear mucus which was normal.

Intestines: Showed normal meconium.

Cultures:

Heart's blood,	negative
Lung,	negative
Spleen,	negative
Liver,	negative
Stomach contents,	negative
Small intestine,	negative
Large intestine,	negative
Meconium rectum,	negative

Guinea pig 2983: Injected with extract from lung, liver, spleen; killed April 15, 1923; negative.

Guinea pig 2984: Injected with stomach contents; killed April 15, 1923; negative.

Smears:

Lung,	negative
Stomach contents,	negative

The only other calf deserving mention was from no. 418. This calf was expelled with the placenta and the umbilical vessels remained intact. Birth occurred about 5 A.M. and the calf was not found by the attendant until 6 A.M. It was alive but very dull. The umbilical vessels were severed and the calf died about one-half hour later.

The following examination and notes were made of this calf:

Sex: Female.<sup>69</sup>

Color: Black and white.

Born: February 2, 1923, to dam 418.

Externally: Normal.

Internally: Tissues appeared normal.

Lungs: Perfectly inflated.

Stomachs: Filled with a faintly clouded mucus, which was apparently normal.

Intestines: Showed normal meconium.

Cultures:

Heart's blood,	negative
Lung,	negative
Spleen,	negative
Liver,	negative
Stomach contents,	negative
Small intestine,	Gram negative slender rod; <i>B. coli</i>
Meconium rectum,	negative

Blood serum: Negative to the agglutination test.

Guinea pig 2897: Injected with extract from lung, liver, spleen; died February 13, 1923; *B. coli* in heart's blood; lungs congested.

Guinea pig 2898: Injected with stomach contents; killed March 19, 1923; negative.



It will be observed (table 2) that all of the placentae of Group I were negative for *Bacterium abortum*, while samples of the colostrum from four of the animals contained the organism. This suggests that vaccinated animals are not very liable to expel the organism from the genital tract at parturition following vaccination even when exposed to severe infection during pregnancy. These experiments confirm the fact that in persistent carriers, the udder is the seat of the infection. No conclusion can be drawn, however, as to whether the udder infection in these four cases resulted from the vaccination or from the infection by ingestion. It should be observed in this connection that none of the animals of Group III which were vaccinated only, eliminated *Bacterium abortum* with their colostrum or placentae.

#### *History of Second Pregnancy of Animals of Group I.*

After the first pregnancy in these animals, one, no. 407, died and two others, nos. 421 and 424 were killed. This left 17 animals in the group for study in the second pregnancy, three of which had failed to get with calf the first year. Two of these, nos. 405 and 415, were successfully bred the second year. The remaining animal, no. 428, we were unable to get with calf. Her genital tract clinically seemed to be normal. She came in heat regularly and from May 10, 1923, to February 13, 1924, she was bred ten times without result and was slaughtered February 18, 1924.

The breeding and calving history of the second pregnancy in this group, together with guinea pig inoculations of the placentae and colostrum, are given in table 2.

Three of the 17 animals, 403, 410 and 418, have not as yet calved the second time, but are definitely with calf.

Only two animals, nos. 4 and 408, in the group eliminated *Bacterium abortum* at the termination of the second pregnancy, whereas four had done so at the termination of the first pregnancy. In both cases it was in the colostrum. These animals, nos. 4 and 408, had given a similar result in their first pregnancies. No. 421, a third animal showing infected colostrum in the first pregnancy, had been killed shortly after the first parturition. The remaining animal, no. 426, in this group eliminating *Bacterium abortum* at the end of the first pregnancy, was negative in the second.



## EXPERIMENTAL DATA ON ANIMALS OF GROUP II

On February 7, 1922, the 13 animals of Group II, A and B, were given a final examination for pregnancy preparatory to placing them in the far east pasture (fig. 1) with the two bulls. One animal, no. 401, was found on rectal examination to be in early pregnancy, a fact not recognized at the time of purchase. One dairy animal, no. 2060, was definitely known to be pregnant to the dairy bull. The remaining eleven were not pregnant. Two other animals were added later to make fifteen, the desired number.

On April 10, 1922, the bulls were taken from this group. During the sixty-two days that they were with them, eight of the eleven open animals became pregnant. Nos. 183, 2317 and 430 did not become pregnant and were later bred to the dairy bull, conceiving without difficulty. It is probable that they did not come in estrum during the sixty-two day period since feed conditions in the pasture were not very good and the weather was cold and rainy.

On June 24, 1922, the ten animals of Group II-A were examined per rectum and found to be definitely pregnant. Nos. 183, 430 and 2317 were at the dairy for breeding to the dairy bull and were later returned with nos. 2298 and 436 to constitute the five association animals of Group II-B.

The ten pregnant animals of Group II-A were moved on this date from the far-east pasture to the road and east pastures with the animals of Group I.

They were kept corralled from June 24 to 26 to control their water supply in the hope they would drink from the watering-trough the infectious material to be given them on the latter date.

These animals were infected on June 26 in the identical manner as those of Group I and were turned into the east pasture with them the next day.

On July 10, fourteen days after infection, blood was drawn from these animals and with the possible exception of no. 401, all gave a positive agglutination test, although they had all given continuously negative reactions prior to the time of infection on June 26. This indicated that they had been infected with the *Bacterium abortum* by the method used (see table 1).

TABLE 3. PARTURITION HISTORY OF TEN COWS IN GROUP II AFTER INFECTION BY INGESTION, JUNE 26, 1922

Number	Aborted	Calved	Placenta		Fetus		Colostrum	Guinea pig inoculation for Bacterium abortum	
			Smears	Guinea pig	Cultures	Guinea pig	Guinea pig		
2060	.....	July 6, 1922	—	.....	.....	.....	—	—	No sample taken
401	.....	July 23, 1922	+	+	.....	+	+	—	—
2181	Aug. 21, 1922—5 months fetus.....	.....	+	+	.....	+	.....	+	—
2180	Sept. 2, 1922—6 months fetus.....	.....	+	+	+	+	+	+	—
429	Sept. 7, 1922—6 months fetus.....	.....	.....	+	+	+	+	+	—
416	Sept. 7, 1922—6 months fetus.....	.....	+	+	+	+	+	+	—
30	Sept. 10, 1922—5 months fetus.....	.....	—	+	—	—	+	+	—
431	Sept. 30, 1922—5 months fetus.....	.....	—	+	+	+	+	—	—
2312	.....	Dec. 3, 1922	—	—	—	—	—	Dry—advanced pregnancy	Pregnant—No sample taken
26	.....	Dec. 3, 1922	—	—	—	—	—	Dry—advanced pregnancy	Pregnant—No sample taken

*History of First Pregnancy of Infected Animals, Group II-A.*

Table 3 gives data on the existing pregnancy in these ten control animals at the time infection was given by the mouth.

It will be observed from examination of this table that six of the ten animals aborted from 56 to 86 days following the infection. No. 2060 calved normally ten days after the infection, which was too soon for it to have caused abortion. No. 401 calved 27 days after the infection—the calf was weak but lived. The placenta was retained and on removal and examination, abortion organisms were found in great numbers in smears and cultures, and inoculated guinea pigs were positive. They were also present in the colostrum. The existing pregnancies of nos. 2312 and 26 were apparently not affected by the infectious material and both animals calved normally on the same date, 160 days after the infection. The examination of the agglutination reaction of these two animals (table 1) shows quite definitely that they became infected but overcame it and remained entirely negative to the agglutination test.

Two animals in this group, nos. 2060 and 401, were much farther advanced in pregnancy at the time of infection than any animals in Group I. The six animals that actually aborted, however, were only about one month farther advanced than a number of animals of Group I. The bulls were with the animals of Group II from February 7 to April 10 and then turned with those in Group I, a number of which were bred during April. We do not think, therefore, that this difference in the period of gestation had any marked effect on the results obtained.

*History of First Pregnancy of Association Animals Group II-B.*

Four of these animals bred to the dairy bull had been at the University Dairy during the infection period and the fifth, purchased in early pregnancy, was not brought on the premises until August, 1922, with the animals for Group IV. They were added to the other animals of Groups I and II on the following dates: Nos. 183 and 2298 on July 25, 1922; nos. 2317 and 430 on August 10, 1922; and no. 436 on September 26, 1922.

The parturition data on these animals are given in table 2.

The six abortions in the ten infected controls of Group II-A, actually took place between August 21 and September 20, 1922. The association animals were in direct contact with them during all of



this period except no. 436, which was added six days after the last abortion occurred. This was the only animal of the five that escaped infection although all of them carried their calves to term.

In studying the agglutination tests (table 1) of these animals, it is interesting to note how the agglutination titre of no. 2298 gradually increased and that of no. 183 remained entirely negative. This latter animal furnishes an example of how the agglutination test may fail to detect a spreader of the organism. No. 430 gave very slight indication of reaction to the agglutination test and no. 2317, although showing much better evidence of infection in the tests made November 10, 1922, March 23 and July 11, 1923, did not at any time develop a definitely positive reaction.

#### *History of Second Pregnancy of Animals, Group II-A and B.*

Following the first pregnancy in this group all of the animals were successfully bred the second time except no. 2060. This animal had previously calved July 6, 1922, ten days after receiving the infectious material by the mouth and too soon for abortion to have occurred. Thereafter although she came in heat frequently and was bred on nine occasions between April 23, 1923, and January 25, 1924, she failed to get with calf and was killed February 28, 1924. Her genital tract at time of slaughter was apparently normal. The examination of her tissues yielded negative results.

No. 431 died October 22, 1923, from traumatic pericarditis when five months pregnant. The vagina was ligated and the uterus removed with enclosed fetus. This material was brought to the laboratory and the following examination made:

Fetus: Five months gestation. Normal.

Cultures:

Cotyledons

Heart blood

Lung

Liver

Spleen

Stomach contents

Intestinal contents

Meconium rectum

All cultures developed a growth of a Gram positive, sporulating rod (*B. subtilis*)

Guinea pigs:

3428, 3429, injected with placental extract.

3424, injected with meconium rectum. .

3425, injected with lung, liver and spleen extract.

3426, injected with stomach and intestinal contents.

The guinea pigs were killed December 11, 1923, and all were found to be in a normal condition.

The remaining eight infected control animals (A) that had received infectious material and the five association animals (B) carried their calves to term.

Nos. 26 and 2312 of the infected controls (A) again failed to show *Bacterium abortum* in the colostrum or placenta at this parturition. The colostrum of all the other six was positive. Of these only no. 401, the animal to calve twenty-seven days after the infectious material was given in 1922, showed infection of the placenta at the second parturition.

The five association animals (B) were negative except the colostrum of no. 2298. It is interesting that the single animal in this group that remained a carrier of the infection was the only one to show a definitely positive blood reaction (see table 1).

### EXPERIMENTAL DATA ON ANIMALS OF GROUP III

On February 7, 1922, the animals of this group were vaccinated in the same manner as those of Group I and placed in the road pasture with them. They were affected by the vaccination in a similar manner to the animals of Group I.

On March 10, thirty-one days after vaccination, the following condition was found on examination of the injected area in these animals:

- No. 402. Abscess had opened naturally.
- No. 406. Enlarged gland.
- No. 413. Enlarged gland.
- No. 419. Enlarged gland.
- No. 2297. Enlarged gland.
- No. 2313. Abscess had opened naturally.
- No. 2315. Enlarged gland.
- No. 2318. Enlarged gland.
- No. 2319. Normal.
- No. 2321. Normal.

These animals at the time were not in such good condition as the thirteen controls of Group II.

On February 21, 1922, fourteen days after vaccination, blood samples were taken from these animals and all gave a positive reaction to the agglutination test (see table 1).



The breeding of this group was begun April 10, 1922, sixty-two days after vaccination, when the bulls were removed from Group II and kept corralled so that breeding dates could be secured. On the night of April 10 the bulls were turned out with the cattle and no. 413 was bred. The animals came in heat slowly although they had apparently entirely recovered from the effects of the vaccination. The following breeding took place:

Bull 412 bred on April 23, 1922, to no. 2297.

Bull 411 bred on April 27, 1922, to no. 406.

While this breeding was going on, the animals of this group were still with those of Group I and were on good green feed in the east pasture.

On May 12, 1922, while examining the genital tracts of the unbred heifers of this group, no. 2318 was found to be about five months pregnant, and on looking up her history, it was found that she had been in the dairy pasture where the dairy bull was given exercise in December, 1921, prior to going into the experiment. She was, therefore, turned into the road pasture with the bred heifers of this group and Group I, although she had been vaccinated on February 7 when about two months pregnant.

On May 18, in the morning, the unbred heifers of Groups I and III in the east pasture got through an open gate into the road pasture with the bred heifers of the same groups. While the attendant was arranging gates in the corral where the animals had been placed with the bulls prior to separating them into bred and unbred groups, bull 412 was observed to breed no. 2318 and she was found to have a vaginal discharge. The laboratory was notified and, upon making a rectal examination, the uterus was found to be empty. The hand could be easily passed into the vagina, which contained a mucopurulent material streaked with blood, a handful of which was withdrawn and placed in a sterile tube. The cervix was open sufficiently to admit two fingers. On massaging the uterus per rectum with the other hand cupped over the cervix, some shreds of tissue with blood clots were expressed and placed in a second sterile tube. An effort was made to demonstrate *Bacterium abortum* by microscopic examination, but it was poor material to use for this purpose and the organism could not be demonstrated. Two guinea pigs, 2221 and 2222, were injected intra-abdominally with a salt solution suspension



of this material. Both were found to be normal when killed and examined August 2, 1922. This animal had definitely aborted between May 12, when she was found to be five months pregnant, and May 18, when she was seen to accept service from the bull. During this period the unbred heifers of this group and of Group I were being corralled twice daily with the bulls, but the bred heifers were not being closely watched. No evidence of the aborted fetus or membranes could be found in a careful search of the road pasture, which was to be expected owing to the fact that the area is hilly and covered with brush-growth in some places. Small predatory animals, including coyotes, infest the area. In examining this animal for pregnancy on May 12, a rectal examination only was made. This animal was continued in the experiment. She did not conceive from the service of May 18, but did so after the bulls were turned with this group in the far-east pasture on June 24, 1922. This pasture, in which this group was placed, had not contained any infected animals and they were left there for the remainder of the experiment, thus being kept free from any infection except that given them in the vaccination, February 7, 1922. At the time they were placed in the far-east pasture, no. 413 was the only one that was pregnant.

#### *History of First Pregnancy of Animals, Group III.*

These animals were examined for pregnancy on September 26, 1922, and nos. 413, 2297, 2318 and 2321 were the only four that were pregnant. Bull 411 was, therefore, left with this group and bull 412 was removed to Group IV-A in the east Rifle Range (figure 1). Later examinations for pregnancy on November 20 and December 29, 1922, and February 26, 1923, showed the above animals to be the only ones that had become pregnant.

On February 5, 1923, bull 411 was in poor condition and was removed from the far-east pasture and placed with Groups I and II which were corralled around the buildings (figure 1) so that he could be fed hay. He was replaced by bull 412, which had been with five heifers in Group IV-A and was in very good condition. On February 12, bull 412 jumped the fence from the far-east pasture to the east pasture where he bred cow no. 20, an infected control in Group II-A, which had aborted September 10, 1922. On that date both bulls were placed in a special corral built for them in the connecting pasture (fig. 1).

The animals in Group III had been in continuous association with one or both bulls from April 10, 1922, to February 12, 1923, a period of approximately ten months, and only four of them had become pregnant. The far-east pasture in which these animals were kept is large, rough and hilly, with considerable brush-growth on the hill-sides. They were not, therefore, under very close observation during all of this period. It could have been possible for them to have become pregnant and to have aborted without being observed. We do not feel, however, that this occurred because of the repeated negative examinations for pregnancy made during the period. It is also improbable that abortions occurred in any of the six animals before a diagnosis of pregnancy was made, and did not occur in any of the four animals in which pregnancy was early diagnosed. The results obtained with the animals in Group I further substantiate the improbability of any of those in Group III having aborted. All six of the animals finally became pregnant between May 1 and the end of September, 1923, and calved normally (see table 2). No abortion organisms were found in the colostrum or placentae of any of these animals. The interesting result in Groups I and III is that of the thirty vaccinated animals, only twenty-one were successfully impregnated within a reasonable time. Eight of the nine finally became pregnant. Six of the nine were constantly in association with one or both bulls for ten months and the other three for the period from April 10 to June 24, 1922. Little difficulty was experienced in getting the control animals of Group II bred. The nine in Groups I and III that failed to get with calf had never been pregnant and they were the youngest heifers.

The only difference in the treatment of the animals in Groups I and III from the controls in Group II was that the former received an injection of living *Bacterium abortum* organisms. At the time, it was concluded that this must have been responsible for the failure of these animals to get with calf. Later a somewhat similar experience was obtained in getting the five animals in Group IV-A with calf. Nothing was done to interfere with the breeding of the animals in Group IV-A, and this has caused us to look for other possible explanations.

It has long been recognized that feed conditions play an important role in the development of estrum. Animals on range have fre-



quently been known to have ovulation delayed for many months when feed was poor. Our experimental animals were kept under semi-range conditions and during the dry season the supply and quality of the feed was such that the animals were not kept in very good flesh, even though the natural feed was augmented by alfalfa or grain hay in small amounts.

The green feed conditions during the last two years have not been good on account of shortage of rainfall. The problem of nutrition, therefore, may have played a part in the failure of these animals to get with calf.

### *History of Second Pregnancy of Animals, Group III.*

In this group only four animals became pregnant the first year. Two of these four were killed shortly after calving and their body tissues examined for *Bacterium abortum*. The other two, nos. 2318 and 2321, conceived again promptly and carried their calves to term, thus terminating their second pregnancy at about the time the other six animals in this group were completing their first pregnancy. Guinea pig inoculation of placentae and colostrum from these animals were negative for *Bacterium abortum* (see table 2).

### EXPERIMENTAL DATA ON ANIMALS OF GROUP IV

The eleven animals in this group were assembled in August, 1922, approximately one year after those in the other groups. Group IV-A was placed in the east rifle range and Group IV-B in the rifle range (fig. 1). They were to be bred without any previous treatment to ascertain whether the male would transmit infection to them in the process of breeding.

On September 26, 1922, bull 412 was removed from Group III in the far-east pasture and placed with Group IV-A. From that time to February 5, 1923, he was constantly in the pasture with them, and thereafter they were seen daily for the presence of estrum although the bulls were kept corralled after February 12, 1923. Despite the fact that no previous treatment had been given them, these animals came in heat slowly. Two of them were bred in December, 1922, one on March 24 and again on May 21, 1923, one on May 27, 1923, and the fifth animal not until about August 1, 1923. A period of ten months was therefore required to get these animals with calf. They



all carried their calves to term (see table 2) and calved normally, except no. 437 which died during parturition from a ruptured uterus.

Opportunity was offered to breed only one of the six animals in Group IV-B by the bull after breeding an aborting animal, and this was some months after the abortion occurred. On April 7, 1923, bull 412 bred cow 429, Group II-A, which had aborted on September 7, 1922, and the same date, following this service, was bred to cow 444, Group IV-B, and she became pregnant to this service. This latter animal calved normally January 11, 1924, and the colostrum was free from *Bacterium abortum* organisms. The examination of the placenta was incomplete.

One other animal in this group, 442, was bred January 2, 1923, but at that time none of the aborting animals had been bred. She also calved normally on October 10, 1923.

The other four animals in Group IV-B were not utilized. They were removed January 31, 1924, and used in another experiment.

The animals which calved in Group IV, A and B, were killed and their body tissues examined for the presence of *Bacterium abortum*. The blood reactions of all of these animals remained negative. The result of the test of the blood sample from no. 445, Group IV-A, on July 11, 1923, was not checked until after the sample was discarded and, therefore, was not retested.

#### STUDY OF BODY TISSUES FOR PRESENCE OF BACTERIUM ABORTUM IN ANIMALS FROM ALL GROUPS THAT HAVE DIED OR BEEN KILLED

To date, twenty-four animals have died or been killed: six were from Group I, four from Group II, seven from Group III, and seven from Group IV. Cultures and guinea pig inoculations have been made from the following tissues with negative results for *Bacterium abortum* in every instance:

##### GROUP I.

No. 407. Died December 28, 1922. Atlantal, submaxillary, mediastinal, mesenteric, supramammary, internal iliac, precrural glands, udder, placenta and point of inoculation of vaccine.

No. 424. Killed February 28, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, gastric, mesenteric, renal, supramammary, internal iliac, precrural, preescapular, cervical glands, udder, liver, spleen, uterus and cervix.

No. 421. Killed March 23, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, gastric, portal, mesenteric, supramammary, internal iliac, precrural, prescapular, cervical glands, liver, spleen, uterus and cervix.

No. 428. Killed February 21, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen, and uterus.

No. 405. Killed July 8, 1924. Supramammary glands, udder, uterus and placenta.

No. 25. Killed September 20, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen and placenta.

#### GROUP II.

No. 431. Died October 22, 1923. Supramammary glands and placenta.

No. 2060. Killed February 21, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen and uterus.

No. 26. Killed May 6, 1924. Supramammary glands, uterus and placenta.

No. 2312. Killed May 6, 1924. Supramammary glands, uterus and placenta.

#### GROUP III.

No. 413. Killed May 21, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen, uterus, ovary and point of inoculation of vaccine.

No. 2297. Killed May 21, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen, ovary and uterus.

No. 2313. Killed February 21, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen and uterus.

No. 402. Killed May 13, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, spleen and uterus.

No. 419. Killed May 22, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, spleen and uterus.

No. 2321. Killed May 22, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, and spleen.

No. 406. Killed September 20, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, spleen and uterus.

#### GROUP IV.

No. 437. Died September 26, 1923. Supramammary glands and placenta.

No. 435. Killed November 20, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, gastric, portal, mesenteric, supramammary, internal iliac, precrural, prescapular glands, udder, liver, spleen, ovary and uterus.



No. 442. Killed November 20, 1923. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, gastric, portal, mesenteric, supramammary, internal iliac, precrural, preescapular glands, liver, spleen, ovary and uterus.

No. 444. Killed February 4, 1924. Supramammary glands and uterus.

No. 438. Killed May 13, 1924. Atlantal, submaxillary, retropharyngeal, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, preescapular glands, spleen and uterus.

No. 445. Killed May 13, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, preescapular glands, spleen and uterus.

No. 439. Killed May 22, 1924. Atlantal, submaxillary, retropharyngeal, bronchial, mediastinal, portal, mesenteric, supramammary, internal iliac, precrural, preescapular glands, spleen and uterus.

In order to conserve guinea pigs, which were very difficult to secure when some of the animals were killed, parts of from two to five lymph glands were ground with physiological salt solution and the resulting suspension injected into a single guinea pig. While the findings were all negative, it was, nevertheless, thought desirable to list each animal separately in order to give the reader the essential details of the examinations in each case. The following is the comment which we have to make in regard to the above data:

*Bacterium abortum* not having been found in the cultures, they were recorded as negative. A variety of bacteria was obtained in some of the cultures, but none had significance in the problem under study. One hundred and thirty-six guinea pigs were used. All survived the inoculation and were killed at the end of about six weeks except the four from cow 437, Group IV-A. This animal died during parturition from a ruptured uterus and the guinea pigs were inoculated with tissues from her supramammary glands and placenta. They died in forty-eight hours following the injection.

Cow 421, Group I, which was killed on March 23, 1923, showed *Bacterium abortum* in her colostrum at time of calving on March 6. When slaughtered, the supramammary lymph glands only were removed for examination as it was not known that the colostrum was positive until the guinea pigs inoculated with it were killed on April 25.

In studying the agglutination tests of the blood of these animals (table 1), it will be seen that nos. 407, Group I, and 2060, Group II-A, were the only ones giving a positive reaction at time of slaughter. The examination of the tissues of both of these animals



was rather extensive. Failure to find the organism is either evidence that our examination was not sufficiently searching or that some animals may give a positive agglutination test for weeks or months after they no longer harbor the live organism. In one cow under our observation free from *Bacterium abortum* infection given repeated injections of dead organisms, a positive agglutination test remained for a period of at least nine months. This case is discussed in the attached footnote.\*

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\* The research of Smith<sup>19</sup> and his associates at the Rockefeller Institute of Animal Pathology has demonstrated that *Bacterium abortum* agglutinins do not pass the placental filter. It has also been shown by others that other immune bodies do not pass through the placenta of bovines and some other ruminants. Nevertheless, it is true that in some other species of animals such passage has been demonstrated. It occurred to us that it would be desirable to eliminate the remote possibility of the absorption of agglutinins by *Bacterium abortum* in the fetus and its membranes by demonstrating the failure of their passage in a non-infected, gravid uterus. We, therefore, developed large quantities of these substances in the blood of a cow just prior to parturition without the uterus being infected so that any agglutinins which passed the filter would be demonstrated in the blood of the fetus at birth.

With this object in view, a suspension of *Bacterium abortum* organisms was made. These were killed by heating to a temperature of 60° C. for fifty-eight minutes and raising the temperature to 65° C. for two minutes. After cooling, three glycerine agar tubes were heavily inoculated with the suspension by means of a sterile glass pipette, and no growth was obtained. To further test the sterility of the suspension, guinea pig 3485 was inoculated with 1 mil of the material on November 27, 1923. This guinea pig was killed on January 8, 1924, and found to be free from infection.

One of our dairy cows, no. 2451, in advanced pregnancy, free from *Bacterium abortum* infection and with negative blood reaction, was injected subcutaneously on the left side of the neck with 5 mils of this suspension of dead *Bacterium abortum* organisms on December 3, 1923. Blood taken from the animal on December 8 was still negative. On December 10, 10 mils of the same suspension were injected on the opposite side of the neck. On December 13, blood from this animal gave a positive agglutination test up to 1-100 dilution. On December 17, 5 mils of the same suspension were injected into each of three different places on the right side of the neck. On December 22, blood from this cow gave a complete agglutination in dilutions up to 1-500 and a partial agglutination in a dilution of 1-1000. She calved normally on December 23 at 10 A.M. The calf was prevented from getting colostrum and a sample of its blood was taken as well as one from the dam. One quart of the colostrum was collected from the udder in approximately equal amounts from all four teats. An agglutination test of the colostrum serum was positive in 1-1000 dilution and of the blood serum a partial agglutination was obtained in this dilution, the same as with the sample taken on the previous day. The agglutinins had, therefore, become more concentrated in the colostrum than in the blood. The serum of the calf was completely negative in all dilutions from 1-25 up.

After the samples were taken from the dam and the calf, the latter was allowed to suckle on the evening of December 23, 1923. On the following morning, blood was again drawn from the calf and gave complete agglutination in dilutions up to 1-1000. Thereafter the agglutinin content of the blood of the cow remained sufficiently high to produce complete agglutination in dilutions over 1-100 for at least nine months. Smith and Little<sup>18</sup> found a persistence of agglutinins produced by the injection of heat-killed organisms in presumably uninfected but exposed heifers for a period of 6½ to 7 months.

ELIMINATION OF BACTERIUM ABORTUM IN MILK OF COWS VACCINATED  
WHILE IN LACTATION

In the series of experiments which has already been discussed, all of the animals that were vaccinated received the injection when they were not in lactation. Opportunity was therefore not afforded to make a study of their milk until the following parturition, almost a year after the live abortion organisms had been administered. At this time it was found that only four of the vaccinated animals eliminated the organisms in their milk and all of them were in Group I which received heavy infection orally in addition to the vaccination.

In order to get information on the question of the elimination of the organisms in the milk of cows that were lactating at the time of vaccination and other questions of importance in the study of the immunity and carrier problem, a second series of experiments was started in December, 1923, with the animals in the University Dairy herd. These animals constituted a particularly valuable group for this purpose because they were known to have been free from *Bacterium abortum* infection for a period of over three years.

Schermer and Ehrlich<sup>17</sup> in an effort to answer the above question inoculated subcutaneously each of three cows with the growth of a slant agar culture of *Bacterium abortum* and at the end of one, two, three and five weeks respectively, milk was taken from each of the animals. It was centrifuged and cultured and in no case did they recover the organism. They also inoculated subcutaneously one cow with five agar tube cultures showing good growth and at the end of seven and thirteen days respectively, milk samples were collected and injected into guinea pigs. Blood samples were taken from the guinea pigs periodically and always showed, according to the authors' statement, a titer under 1-100. The guinea pigs were killed nine weeks after the inoculation and showed no lesions of abortion. Cultures made from their organs were free from *Bacterium abortum*.

Zeller<sup>22</sup> also concerned himself with this question and performed the following experiments. One cow was inoculated subcutaneously behind the shoulder with 10 slant agar cultures in 20 mls of salt solution. On seventeen occasions, from one to eighty-nine days after the inoculation, samples of blood, milk, saliva, feces and urine were cultured for the presence of *Bacterium abortum* with negative results



in all cases. The animal was killed 128 days after the injection, and cultures from the internal organs gave negative results. Inoculation of four guinea pigs with the spleen and udder also resulted negatively.

Another cow was inoculated with 20 slant agar cultures in 30 mils of salt solution. On sixteen occasions, from three to sixty-seven days subsequently, the same materials as those taken from the previous cow were cultured with negative results. On each of these occasions one guinea pig was inoculated with cream and sediment of milk from all four quarters. At the end of two months the guinea pigs were killed and found free from infection. Extensive cultures from their body tissues were negative and their blood gave a negative agglutination test.

A third cow was inoculated as above and on twenty-one occasions, from one to sixty-five days subsequently, milk, blood, etc., were cultured and one guinea pig inoculated with the milk. All cultures and guinea pigs remained free from *Bacterium abortum* infection.

A fourth cow, not in lactation, was inoculated with 10 agar slant cultures. At various intervals samples of all the previously mentioned materials except milk were collected from the animal and inoculated into cultures and guinea pigs with negative results. At 161 days after the injection the animal was slaughtered and her tissues cultured with negative results. Guinea pigs inoculated with the material from the spleen, uterus, ovary, udder and supramammary lymph glands were negative.

In our own investigations of this question during the past year sixteen head of non-pregnant milking cows have been vaccinated. The live abortion organisms used in this vaccine were the same as those used in the first series of experiments except that strain 101 was replaced by strain 150, which had been more recently isolated (September 25, 1922) from an aborted bovine fetus. These organisms were grown on solid media only. The quantity of organisms injected was one-half the number used in the first series or less.

The material was prepared by having the opacity of the suspension equal to a two billion meningococci standard in place of a four billion as used in the first series of experiments. This showed a Gates<sup>7</sup> reading of 1.5 cm. With the plate culture method of counting, this suspension was found to contain from 4.47 to 6.8 billion organisms per mil. Hagan<sup>9</sup> estimated by the plate method that a



*Bacterium abortum* suspension of 2.4 cm. Gates' reading varied between 4.5 and 13 billion organisms per mil. When we diluted our suspension of 1.5 cm. Gates' reading to equal 2.4 cm. and used Hagan's average of 8.74 billion per mil, we found our suspension of 1.5 cm. was closer to his lower count.

To make 20 mils of this concentration required about one slant agar culture of the organism.

These various methods of estimating the number of organisms are given in order that our dose may be compared with that used by other workers. The number of mils of the suspension given to each cow varied and is given in table 5.

The vaccine was administered subcutaneously at several places and in some cases additional dilutions were made to obviate local swelling and abscess formation as far as possible. Great care was used in vaccinating the animals to prevent any contamination of the premises. In four of the cases local swellings developed into abscesses. These animals were isolated during the surgical removal and healing of the abscessed areas. Eighteen controls have been in association with the vaccinated animals and that no transmission of the infection has so far taken place has been determined by regular guinea pig inoculations with milk samples and monthly agglutination tests of blood samples.

After the vaccination, samples of milk were collected, in some cases daily for three days and in all cases weekly for two or three weeks. Samples of milk from all the animals in the dairy barn were tested for *Bacterium abortum* in a routine manner at intervals of one to two months.

The samples of milk were collected, before the regular milking, in sterile, wide-mouthed bottles, about one-fourth of the sample being taken from each quarter, with a total of 300 to 400 mils. In some cases a similar sample was collected after the milking machine had been removed and before stripping was started. No particular difference in the *Bacterium abortum* content of the two kinds of samples has been found.

Ten of the sixteen lactating animals that were vaccinated have given off *Bacterium abortum* in their milk. In no case has this organism been definitely isolated during the first three days after vaccination. From the end of this period to the seventh day, no samples

TABLE 4.—TEMPERATURE REACTIONS OF VACCINATED ANIMALS IN SECOND SERIES OF EXPERIMENTS WITH RESULTS OF MILK EXAMINATIONS FOR THE PRESENCE OF BACTERIUM ABORTUM

No. of animal	Date of vaccination	Temperature when vaccinated	Amount of vaccine	Temperature for five days subsequent to vaccination					Weekly milk examinations following vaccination								
				106.2	105.8	104.6	101.8	102.8	Date	Number of guinea pigs inoculated	Result	Date	Number of guinea pigs inoculated	Result	Date	Number of guinea pigs inoculated	Result
2399	Dec. 18, 1923	102.6	10 mls	106.2	105.8	104.6	101.8	102.8	Dec. 24	3519	Both Positive	Dec. 31	3529	Both Positive	.....	.....	.....
2400	Dec. 18, 1923	102.2	10 mls	106.0	106.2	105.4	105.4	104.0	Dec. 24	3520	Both Positive	Dec. 31	3530	Both Positive	.....	.....	.....
2401	Dec. 18, 1923	102.0	10 mls	104.5	106.0	104.4	105.1	104.2	Dec. 24	3521	Negative	Dec. 31	3531	Both Negative	.....	.....	.....
2421	Dec. 18, 1923	103.0	10 mls	106.0	107.4	105.4	105.0	103.8	Dec. 24	3522	Both Negative	Dec. 31	3532	Both Negative	.....	.....	.....
2154	Jan. 5, 1924	101.6	10 mls	106.7	106.0	103.8	103.8	101.6	Dec. 24	3523	Both Negative	Dec. 31	3533	Both Negative	.....	.....	.....
									Dec. 24	3524	Both Negative	Dec. 31	3534	Both Negative	.....	.....	.....
									Dec. 24	3525	Both Negative	Dec. 31	3535	Both Negative	.....	.....	.....
									Jan. 14	3526	Both Negative	Jan. 23	3536	3550 died Jan. 24	.....	.....	.....
									Jan. 14	3547	Negative	Jan. 23	3549	3549 positive	.....	.....	.....
										3548	Negative		3550		.....	.....	.....
440	Jan. 31, 1924	101.4	20 mls	106.8	106.2	104.2	102.2	103.2	Heifers	not milking.		Mar. 21	3754	Positive	Mar. 28	3762	Positive
441	Jan. 31, 1924	101.6	20 mls	106.6	105.8	103.8	105.0	104.0									
2055	Mar. 8, 1924	101.2	15 mls	106.8	106.6	105.0	104.6	105.0	Mar. 14	Milk samples							
2142	Mar. 8, 1924	101.4	15 mls	106.6	105.8	104.8	103.2	104.8	Mar. 14	(Cultured)		Mar. 21	3755	Negative	Mar. 28	3763	Positive
2405	Mar. 8, 1924	101.2	15 mls	106.4	106.0	105.2	103.6	103.2	Mar. 14	Negative		Mar. 21	3756	Negative	Mar. 28	3764	Positive
2143	Apr. 14, 1924	102.4	10 mls	103.2	106.8	104.0	102.2	102.4	Apr. 22	3802	Negative						
2504	Apr. 14, 1924	102.0	10 mls	106.0	105.8	105.0	103.8	102.6	Heifer	not milking.							
2144	May 19, 1924	102.6	10 mls	106.0	106.0	105.8	103.8		May 28	3896	Negative	June 2	3899	Negative	June 9	3903	Negative
1441	May 19, 1924	102.4	10 mls	105.6	107.0	106.0	102.6		May 28	3895	Negative	June 2	3898	Positive	June 9	3904	Negative
2062	July 7, 1924	102.4	10 mls	105.6	106.4	105.4	103.2		July 14	3933	Negative	July 22	3979	Negative	July 28	3901	Positive
2316	July 7, 1924	102.6	10 mls	107.0	106.2	105.2	105.6		July 14	3934	Negative	July 22	3979	Negative	July 28	3902	Negative
										3951	Negative	July 22	3980	Negative	July 28	3981	Negative
										3952	Negative	July 22	3980	Negative	July 28	3982	Negative
2304	Aug. 25, 1924	101.6	10 mls	106.4	106.6	104.8	103.2	101.2	Sept. 1	4041	Negative	Sept. 10	4047	Negative	Sept. 15	4048	Positive
1438	Oct. 14, 1924	101.8	10 mls	105.6	105.2	104.6	103.6	104.3	Oct. 20	4129	Negative	Oct. 28	4133	Negative	Nov. 4	4141	Negative
2503	Oct. 14, 1924	102.4	10 mls	105.8	106.0	104.7	102.2	102.2	Oct. 20	4130	Negative	Oct. 28	4131	Negative	Nov. 4	4142	Negative
													4032				



TABLE 4.—TEMPERATURE REACTIONS OF VACCINATED ANIMALS IN SECOND SERIES OF EXPERIMENTS WITH RESULTS OF MILK EXAMINATIONS FOR THE PRESENCE OF BACTERIUM ABORTUM—(Concluded)

Monthly to bimonthly examinations of milk following vaccination												
No. of animal	Date of first sample	Num-ber of guinea pigs inoc-ulated	Result	Date of second sample	Num-ber of guinea pigs inoc-ulated	Result	Date of third sample	Num-ber of guinea pigs inoc-ulated	Result	Date of fourth sample	Num-ber of guinea pigs inoc-ulated	Result
2399	Feb. 14	3628	Both Positive	Apr. 14	3789	Positive	May 28	Dry	.....	July 14	Dry	.....
2400	Feb. 14	3629	Negative	Apr. 14	3783	Negative	May 28	3881	Negative	July 14	3953	Negative
2401	Feb. 14	3617	Negative	Apr. 14	3788	Negative	May 28	3885	Negative	July 14	3954	Negative
2421	Feb. 14	3627	Negative	Apr. 14	3791	Negative	May 28	3886	Negative	July 14	3955	Negative
2154	Feb. 14	3633	Both Positive	Apr. 14	3780	Positive	May 28	3880	Positive	July 14	3956	Negative
440		3608									3960	Positive
441		3609									3943	Positive
2055				Apr. 14	3796	Positive	May 28	3891	Positive	July 14	3944	Positive
2142				Apr. 14	3799	Positive	May 28	3894	Negative	July 14	3931	Positive
											3932	Positive
											3937	4004
											3938	Positive
2405				Apr. 14	3798	Positive	May 28	3893	Negative	July 14	3957	Negative
2143				Apr. 14	3795	Negative	May 28	3890	Negative	July 14	3958	Negative
2504											3939	Positive
2144							May 28	3896	Negative	July 14	3940	Positive
											3941	Negative
											3942	Positive
1441							May 28	3895	Negative	July 14	3921	Negative
2062											3922	Negative
											3933	Negative
2316											3934	Negative
											3951	Positive
2304											3952	Negative
1438												Positive at the 14th day
2503												Negative through-out
												Positive at the 50th day
												Positive at the 21st day

NOTE.—Each time the five monthly to bimonthly samples were taken, milk from every cow was tested. The unvaccinated animals numbering from eleven to twenty were always negative for Bacterium abortum.



were taken. In one animal the milk was definitely positive at the end of the first week. The milk of the remaining nine was demonstrated to be positive from the thirteenth to the fifty-fifth day.

The data on these milk examinations so far as it has been carried at the present time are given in table 4.

### DISCUSSION

Throughout the presentation of the paper, comment on the experimental data is made. The detailed work involved in the study of the problem under consideration is difficult to present in a manner to be easily followed. To obviate this, we have placed the data as far as possible in tables. On account of the care used in collecting and examining the experiment animals and the keeping of the various groups in pastures so situated that infection had no opportunity to spread from group to group, we have given the exact procedures that were carried out throughout the work for those who desire to study or criticize them.

The data show that of the twenty animals in Group I, seventeen became pregnant in from 70 to 137 days after the vaccination. From two to sixty-nine days after their impregnation these animals were given severe infection orally. All of them, except no. 407, which was accidentally killed late in gestation, dropped calves at the termination of a normal gestation period, ranging in these particular cows from 271 to 292 days. In one animal, no. 2305, the calf was born dead at the end of a 278-day gestation period. The placentae in all of these cases were free from *Bacterium abortum* infection.

In direct association with these animals were the ten controls of Group II-A, which were infected but not previously vaccinated. The infection took place at the same time and in the same manner as that given to the animals of Group I. Six of the animals in Group II aborted fifty-six to eighty-six days after the infection given orally. The placenta in every one of these cases showed the presence of *Bacterium abortum*. In addition this organism was found in the placenta of one of the remaining four animals in this group which calved twenty-seven days after infection was given. In regard to the remaining three animals, one calved ten days after the infection was given and the other two 160 days afterwards. The placentae of all three were free from *Bacterium abortum* infection.

These results justify the statement that the subcutaneous injection of living *Bacterium abortum* in the animals of Group I protected their fetuses and membranes from the same exposure to infection that caused the infection of seven out of ten animals in Group II. The animals of Group I were not so far advanced in pregnancy as those of Group II when the infection was given orally. However, in addition to the original infection, they were exposed to the aborted fetuses and discharges from the animals of Group II that aborted between August 21 and September 20, 1922. This gave them ample opportunity to pick up further infection when they were in the middle of their gestation periods. The thirty-four animals constituting all of Groups I and II, except no. 436, were penned every night in a corral of about one-half acre, and fed hay placed on the ground. Four of the abortions took place here. Further evidence that this was a natural exposure is demonstrated by the fact that all four of the association animals of Group II-B, which were with these animals while the abortions were taking place, picked up the infection although none of them aborted. (See table 2.)

Incidentally, the experiments indicate that abortion infection and premature expulsion of the fetus can be induced by a single exposure to infectious material given orally. The material used for infection consisted of fetal tissues, milk and cultures of *Bacterium abortum*. This was because we desired to give the animals a very severe exposure and to include material from sources that would constitute as nearly as possible the usual means of spreading the organism from the carrier of the infection to the healthy animal. In the mixture there were, of course, organisms other than *Bacterium abortum* present, but the latter were without question responsible for the abortions obtained since the animals of Group I were protected against *Bacterium abortum* only and did not abort.

In the matter of the ability of cultures of *Bacterium abortum* to produce abortion, it appears appropriate at this time to call attention to the fact that we have since our experiments began vaccinated three pregnant animals with live *Bacterium abortum*, although the presence of pregnancy was not known at the time the vaccine was given. All of these animals aborted and in two of the cases where the fetus and membranes were available for study, *Bacterium abortum* was readily recovered. One of these animals, no. 2318, Group III, previously



mentioned, aborted a five months fetus a little over three months after the vaccination. The other two were in the second series of experiments. These animals were heifers and were not examined for pregnancy prior to vaccination as the foreman had no record of their having been bred. They were nos. 2420 and 2424 and were both vaccinated December 18, 1923, when about four months pregnant. No. 2420 aborted March 5 and No. 2424 on March 12, 1924, seventy-eight and eighty-five days respectively after the vaccination.

In a previous publication<sup>10</sup> reporting the progress of these experiments, it was stated, "Nine of the 30 vaccinated animals have up to date (May, 1923) failed to conceive." Since that date, eight of the nine head have conceived and calved normally between February 8 and June 26, 1924. It was at that time also concluded that "no other explanation can be offered for this sterility except the vaccination." In the fall of 1922 we extended our experiments to include the animals of Group IV, an untreated and non-infected group. The five animals in Group IV-A required a period of ten months for all of them to conceive. The effect of poor feed conditions entered here as a factor. While at this time we cannot entirely absolve the vaccination from being a causative factor in the failure of the nine animals of Groups I and III mentioned above to conceive, we should not fail to consider that other factors, particularly feed conditions, may have shared in the cause of the temporary sterility.

The blood tests of this fairly large group of animals taken monthly over a period of nearly three years with known time and method of infection and also certain definitely ascertained dates of elimination of *Bacterium abortum* from some of the animals furnishes data deserving comment.

It will be observed from table 1 that the thirty animals in Groups I and III were infected subcutaneously on February 7, 1922, before which time all had been negative to the agglutination test. Fourteen days later all of the animals were definitely positive. On June 26, 1922, the ten animals of Group II-A, all negative to the blood test, were infected orally. Fourteen days later all of the animals, with the possible exception of no. 401, were definitely positive. No blood samples were taken earlier than fourteen days after exposure in these cases. In some other experimental work where five animals were infected orally with cultures of *Bacterium abortum*, two showed



definite reactions at the end of eight days and all at the end of fourteen days. In the vaccination of the animals in the second series of experiments, positive agglutination tests were obtained at the expiration of seven days in several cases. Zeller<sup>22</sup> reports positive agglutination tests in a titer of 1-100, six days after subcutaneous injection. Smith and Little<sup>18</sup> demonstrated positive agglutinations in vaccinated and presumably uninfected, exposed cattle ten days after vaccination. With our cultures it appeared that oral administration was just as effective in producing a positive agglutination titer in the dilutions used as subcutaneous inoculation and that the time required was regularly within fourteen days.

In studying the persistence of the agglutination reaction in animals after a single exposure to infection by subcutaneous inoculation, when non-pregnant, Group III shows that while some of the animals after a period of a few months became negative, others persisted for a longer time even up to one year. The presence or absence of pregnancy intervening during this period seemed to have little effect on the agglutination titer. In comparing the agglutination reactions of the animals of this group with those of Group I which were vaccinated and infected, it will be observed that in a general way they were practically the same with the notable exception of the two animals, nos. 4 and 408 of Group I, which remained eliminators of the organism at the expiration of their second pregnancy. On the other hand, there was a persistence of the agglutination titer in the non-vaccinated but infected animals of Group II-A which continued to harbor and eliminate the organism at the end of the second pregnancy.

In correlating the agglutination titer and the elimination of *Bacterium abortum*, we have attempted to see whether animals discharging these organisms always have a titer of at least 1-100 as strongly suggested by Schroeder and Cotton<sup>16</sup> in their studies of the milk from fifty-six cows of which the blood serum of thirty reacted in dilutions of 1-200 or higher and twenty-six showed an agglutination titer of less than 1-100. Twenty-five of the thirty high reacting cows were eliminating *Bacterium abortum*, while none of the twenty-six low reacting cows were doing so. In work along this line on the University Farm herd at Davis by Hayes and Barger,<sup>11</sup> it was found that the herd contained fifteen reactors to the agglutination test in

TABLE 5. SHOWING REGULAR MONTHLY AGGLUTINATION TESTS NEAREST TO THE DEFINITE DATES WHEN BACTERIUM ABORTUM WAS KNOWN TO HAVE BEEN ELIMINATED FROM ANIMALS OF GROUPS I AND II

Group Number	No. of Animal	Calved or aborted	Agglutination test		Positive milk samples	Agglutination test Nov. 10, 1922	Second calving date	Agglutination test	
I	4	Jan. 23, 1923.....	Jan. 10	Feb. 14			Feb. 22, 1924.....	Jan. 30	Feb. 27
			± ± ± ±	± ± ± ±				± ± ± ±	± ± ± ±
	408	Jan. 25, 1923.....	± ± ± ±	± ± ± ±			Feb. 23, 1924.....	± ± ± ±	± ± ± ±
	421	Mar. 6, 1923.....	Feb. 14	Mar. 23				± ± ± ±	± ± ± ±
II	426	Mar. 24, 1923.....	± ± ± ±	± ± ± ±			Mar. 19, 1924.....	Feb. 27	Mar. 25
			± ± ± ±	± ± ± ±				± ± ± ±	± ± ± ±
	401	July 23, 1922.....	July 10	Aug. 10			Feb. 28, 1924.....	± ± ± ±	± ± ± ±
			± ± ± ±	± ± ± ±				± ± ± ±	± ± ± ±
A	2181	Aug. 21, 1922.....	Aug. 10	Sept. 12	Nov. 10, 1922.....	± ± ± ±	Feb. 19, 1924.....	Jan. 30	Feb. 27
			± ± ± ±	± ± ± ±				± ± ± ±	± ± ± ±
	2180	Sept. 2, 1922.....	± ± ± ±	± ± ± ±	Nov. 10, 1922.....	± ± ± ±	Apr. 27, 1924.....	Mar. 25	Apr. 30
			± ± ± ±	± ± ± ±				± ± ± ±	± ± ± ±
B	429	Sept. 7, 1922.....	± ± ± ±	± ± ± ±			Jan. 3, 1924.....	Dec. 27	Jan. 30
			± ± ± ±	± ± ± ±				± ± ± ±	± ± ± ±
	416	Sept. 7, 1922.....	± ± ± ±	± ± ± ±	Nov. 10, 1922.....	± ± ± ±	Nov. 13, 1923.....	Oct. 30	Nov. 27
			± ± ± ±	± ± ± ±				± ± ± ±	± ± ± ±
C	20	Sept. 10, 1922.....	± ± ± ±	± ± ± ±	Nov. 10, 1922.....	± ± ± ±	Nov. 20, 1923.....	± ± ± ±	± ± ± ±
	431	Sept. 20, 1922.....	Sept. 12	Oct. 10				± ± ± ±	± ± ± ±
			± ± ± ±	± ± ± ±				± ± ± ±	± ± ± ±
	183	Feb. 16, 1923.....	Feb. 14	Mar. 23				± ± ± ±	± ± ± ±
D	430	Apr. 27, 1923.....	Apr. 17	Mar. 12				± ± ± ±	± ± ± ±
			± ± ± ±	± ± ± ±				± ± ± ±	± ± ± ±
	2208	Feb. 3, 1923.....	Jan. 10	Feb. 14			Feb. 25, 1924.....	Jan. 30	Feb. 27
			± ± ± ±	± ± ± ±				± ± ± ±	± ± ± ±
E	2317	Mar. 16, 1923.....	Feb. 14	Mar. 23				± ± ± ±	± ± ± ±
			± ± ± ±	± ± ± ±				± ± ± ±	± ± ± ±

NOTE.—For explanation of symbols see table 1.



dilutions of 1-100 or greater and seven of them were discharging *Bacterium abortum* in their milk. There was, however, one animal with a negative agglutination test that eliminated the organism in the milk. Fitch and Lubbehusen<sup>6</sup> found that fourteen of forty-eight animals that reacted to the agglutination test eliminated *Bacterium abortum* in the milk. The blood serum of these fourteen always had an agglutination titer as high as 1-100 at the time the milk specimen was received. We find that the data in tables 2 and 3 show that there have been twenty-nine occasions in which *Bacterium abortum* has been demonstrated to be eliminated from the bodies of fifteen of the animals in Groups I and II. At twenty-one of these periods, the agglutination titer was positive, at two it was very suspicious and at the remaining six it was practically negative.

The principal failures in this respect occurred in three of the association animals of Group II-B. However, none of these three animals remained permanent carriers. One animal in this group, no. 2298, was negative at the time of the first elimination of *Bacterium abortum* but remained a carrier and soon became definitely positive to the blood test and continued so to the end of the second pregnancy when she again eliminated the organism. The details of these observations we have prepared in table 5.

It has been demonstrated in these experiments and from other observations we have made that *Bacterium abortum* is eliminated from the udder or genital tract of cows when their agglutinating titer is under 1-100 or even entirely negative. It is well to recognize this limitation of the agglutination test in efforts to use it in controlling the disease. Nevertheless, in view of the fact that the eradication of the disease by the blood test method is recommended only in herds where the percentage of reactors is small, the law of proportion tends to reduce the importance of this limiting factor to a great extent.

In regard to the study of the body tissues of animals that have been killed in the experiments, we have nothing to add here to the discussion given in the text of the article.

It will be observed that although the fetuses and membranes of the animals in Group I were protected from infection, the organisms remained alive in the udder and were eliminated with the colostrum of four, or 25 per cent, of the animals that completed their gestation periods. Three of these four animals completed a second gestation



and in two of them it was again demonstrated in the colostrum at this time even though the membranes remained again uninfected and the fetuses were born at term and normal.

All of the animals in the first series of experiments were specially collected for the experiment and none of them were in lactation at the time of the vaccination. They did not offer an opportunity, therefore, to study the mammary secretion for the presence of the organism immediately after vaccination. This was afforded, however, when the second series of experiments was started to include the milking animals in the University Dairy. Immediately after the vaccination of these animals was under way and a study of their milk made, no difficulty was experienced in getting positive results. There was a total of sixteen lactating animals vaccinated and the milk of ten of these has up to date become positive in from seven to fifty-five days subsequent to vaccination. This has been contrary to the experience of other investigators of this disease, notably Schermer and Ehrlich and Zeller, whose work has already been reviewed. Its repeated occurrence in our animals following several different vaccinations leaves no doubt of the probability of *Bacterium abortum* organisms injected under the skin of the neck in lactating animals in the form of vaccine being discharged from the udder with the milk secretion in from one to several weeks after the vaccination. Whether they will remain permanently located in the udder in such cows, we have not had time to ascertain, but reference to table 4 will show that the organisms will continue in this location for a considerable time and to the end of the lactation period in which the animals were vaccinated. This further emphasizes the fact generally accepted that *Bacterium abortum* vaccine should never be used in uninfected herds—also, that there is danger in bringing recently vaccinated animals into abortion-free herds.

It will be observed in the first series of experiments that a marked reaction followed the administration of the vaccine to the animals of Groups I and III. No temperatures were taken of these animals. In the second series of experiments, temperatures were taken in all cases for several days immediately following the vaccination, and it was found that invariably there was a decided pyrexia. The temperatures are given in table 4. Similar observations were made by Smith and Little<sup>18</sup> and Schermer and Ehrlich.<sup>17</sup> In addition to the

temperature reaction, there was a temporary loss of appetite and reduction in the milk flow.

In the article reference was made to the abscess formation following the vaccination and the isolation of *Bacterium abortum* in the two cases which were studied. In the second series of experiments, we had abscess formation in four cases and in all of them the organism was readily recovered; in three of the animals, it was obtained in pure culture.

Many months were required to get the animals of Groups III and IV-A bred. We did not intend to breed the animals of Groups I and II the second time until all the animals of Groups III and IV-A were bred. Such a long period was required to complete the breeding of the latter groups, that it was necessary to proceed with the second breeding of the first two groups. The bulls were therefore moved back and forth to some extent among all four groups during 1923 and no ill effects were observed from this. The entire series of experiments failed to yield any evidence that the bulls transmitted infection mechanically in the process of breeding or otherwise. However, it must be recognized that good opportunity for the bulls to do so was probably not available. It will be observed in table 2 that the vaccination of Groups I and III occurred on February 7, 1922. The bulls bred the animals of Group I from April 10 to June 24. They were then placed with the animals of Group III. It was not until September 26, nearly seven months after the vaccination, that one of the bulls was placed with the animals of Group IV-A.

Attention is directed to the agglutination tests of bull 411. This bull and bull 412 were placed with the vaccinated Groups I and III on April 10, 1922. While the abscesses on these animals were healed at that time, some lurking infection from the discharges from these areas might have been present in the corral or pasture. The first blood test after that date was made on June 14, 1922, when bull 411 showed a positive agglutination in a dilution of 1-50 and continued to do so at irregular intervals for a considerable period of time afterwards. An agglutination at this dilution is generally considered below the positive point, especially when repeated tests fail to show an increase in the agglutination titer. These may be the normal agglutinins of this animal's blood or there is a possibility of this animal having picked up some infection from the vaccinated animals which he managed to keep in abeyance.



### CONCLUSIONS

1. The value of living cultures of *Bacterium abortum* in preventing abortion in the vaccinated animals when subjected to the identical infection that produced abortion in the control animals was demonstrated.

2. A correlation of the agglutination tests of the animals with the definite periods when *Bacterium abortum* was known to have been eliminated shows that this organism may be discharged from the body without its presence being suspected from the agglutination titer of the blood. This calls attention to the limitations of the agglutination test rather than demonstrates its inapplicability as a means to be used in the control of the disease.

3. It has been demonstrated that in a certain percentage of lactating animals injected with *Bacterium abortum* under the skin of the neck, the organisms so injected, or their progeny, will gain access to the udder and be eliminated with the milk. Vaccinated animals may, therefore, become spreaders of the infectious agent under these conditions and cannot with safety be moved into uninfected herds.

4. Animals that develop sufficient immunity to *Bacterium abortum* infection after vaccination to prevent abortion or disease of the placental tissues may still harbor the living organism and eliminate it from the udder.

5. Non-pregnant animals injected with living *Bacterium abortum* subcutaneously when not in lactation and not exposed to further infection failed to show the presence of the organism in the placenta or colostrum at the termination of the following pregnancy.

6. Animals exposed to *Bacterium abortum* in no other way except by vaccination will continue to give positive agglutination tests in a titer of 1-100 for several months to one year after the injection.

7. Vaccination of virgin heifers may be a factor in retarding their impregnation but this has not been satisfactorily demonstrated.

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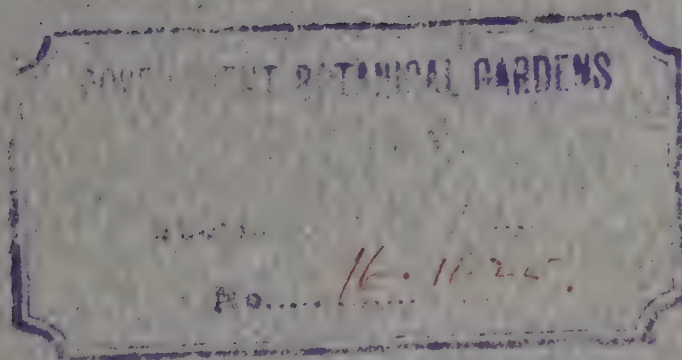
TECHNICAL PAPER No. 20

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A STUDY OF THE CONDUCTIVE TISSUES  
IN SHOOTS OF THE BARTLETT PEAR AND  
THE RELATIONSHIP OF FOOD MOVEMENT  
TO DOMINANCE OF THE APICAL BUDS

BY

FRANK E. GARDNER



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## NOTICE

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This number closes the series known as *Technical Papers*. In the future matter of the same general nature as that which has previously appeared in the Technical Papers will be issued in serial form under the title

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Director California Agricultural Experiment Station.



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FRANK E. GARDNER†

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\* Abridged from a thesis submitted in partial fulfillment of requirements for the degree of Master of Science, University of California, May, 1924.

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## INTRODUCTION

The tendency of most trees to produce branches from the terminal and apical buds, while the more basal buds of the shoot remain dormant, has long been a subject for observation and conjecture. This dominance of the upper buds tends to produce an excurrent type of growth which is striking in a number of fruit trees, particularly in many varieties of the sweet cherry and pear.

It is well known that ringing, wounding, bending, and other practices bring about the growth of buds which normally would remain dormant, though the reason for this response is not known. In the carbohydrate-nitrogen concept, a new aspect of growth has been evolved which may, when more clearly understood, furnish a basis for the treatment of trees in order to bring them into the proper balance of growth and fruitfulness. It is, then, from the standpoint of nutrition, that this study of dominance is undertaken.

Many explanations of the dominance of the apical buds have been advanced; among them Loeb's<sup>15</sup> inhibitor theory which is also sponsored by Reed and Halma.<sup>22</sup> This theory is not free from objectionable features and, moreover, seems to be as incapable of proof as of refutation.

Barker and Lees,<sup>3</sup> while favoring the inhibitor theory, feel that other factors, such as bud strength and sap supply to the buds, play an important rôle.

McCallum,<sup>18</sup> after studying what he considered to be the possible causes of dominance, concluded that "physiological correlation" is the factor responsible and that its effect probably acts along protoplasmic connections.

Child and Belamy<sup>5</sup> carry this conception still further by stating that the difference between the tip and the base of a shoot is a difference in the rate of fundamental metabolic reaction, such differences being associated with differences in protoplasmic condition and appearing in the form of gradients from the tip to the base. To these differences they apply the term, "physiological gradients."

In order to support their theory that dominance is not a matter of nutrition, these writers distinguish between two aspects of the relation of parts. The first is concerned with the conditions which permit or prevent the initiation of growth in a subordinate part, and the second,

with the amount of growth which may occur after its initiation. They believe that nutritive factors play a large part in the amount of growth, but that there are no reasons for believing that such factors initiate growth.

It is recognized that plants in order to grow must utilize both carbohydrate and nitrogenous foods. That these foods are moved from place to place within the plant is certain, yet there is no conclusive evidence as to what tissues are involved in the movement. However, it may be assumed that elaborated materials are translocated through one or perhaps both of the so-called "conducting elements"; namely, the sieve-tubes or the tracheal tubes.

Atkins<sup>1</sup> view is the usual conception of food movement in plants and is typical of that held by most physiologists. He states that the xylem tissue is essential for upward translocation of foods, but that downward movement of elaborated material is through the phloem and thence, by means of the medullary rays, into the inner portions of the tree for storage.

Curtis<sup>6</sup> departs from the orthodox position on this matter by stating that his experiments with ringing and defoliation show that carbohydrates, at least, move exclusively in the phloem.

Although Curtis conclusively showed that ringing interrupts the upward movement of carbohydrates, and Mason<sup>17</sup> found that downward movement is also checked in this way, it yet does not necessarily follow that translocation of these foods is restricted to the phloem. Dixon<sup>9</sup> points out that this interpretation rests on the fallacy of supposing that the removal of a ring of bark leaves the outer layers of xylem uninjured. It is the outer wood which he believes to be functional in food conduction, whereas the whole cross-section of xylem is available for water transport. It is his opinion that carbohydrates not only move upward through the xylem but that they also descend through this tissue.

Although the question of what tissues are involved in food conduction may at first appear unrelated to the matter of dominance of the apical buds, there is a possibility that buds develop into lateral branches as a result of food movement and its accumulation in some particular tissue.

In this study new evidence was sought which might lead to a better understanding of the nature of dominance in shoots and of what tissues are concerned in the conduction of foods.



## METHODS

*Influencing Nutritive Conditions.*—In order to influence the nutritive conditions of pear shoots and to cause buds to develop in lateral branches, several methods were employed:

1. Early in July and in succeeding months, shoots of the current season's growth were bent over through an angle of at least ninety degrees and tied in that position.

2. Portions of shoots were defoliated at intervals throughout the summer. In some cases the leaves were removed from the terminal half of the shoot, and in other cases from the basal half.

3. Shoots were ringed, and in some cases also defoliated either above or below the ring.

4. Shoots were headed-back on July 20 and 31.

It was planned to correlate the growth responses of the foregoing treatments with the carbohydrate and nitrogen contents of the shoots, but this phase of the problem was rendered unnecessary by the appearance of a contribution by Harvey<sup>12</sup> which contained complete tables of the analyses here contemplated. While it is perhaps unfortunate that his analyses do not deal with the bark and wood separately (as the proportion of bark to wood varies considerably between the tip and the base of a shoot) still his results, with that exception, are accepted as a working basis in this paper.

*Forcing Solutions through Shoots.*—In order to test the conduction capacity of ringed and bent shoots as compared with normal shoots, a very simple device was used (pl. 2, fig. 2). The height of the water column was four and one-half feet, which supplied a pressure of approximately one-seventh of an atmosphere. The stems were cut and adjusted to the rubber tubing under water to prevent air from getting into the open tracheae. The unit of measurement was the time required for two cubic centimeters of water to pass through the section of the shoot and to accumulate in the graduated stand-tubes.

*Microchemical Determinations.*—Sections were cut twenty microns thick on a sliding microtome. These were tested for starch with a potassium iodide-iodine solution. For sugars, the Flückiger reaction gave good results. It is a sensitive reaction and gives a test which is unmistakable. Care must be taken, however, to wash the



sections in distilled water, both before and after making the test, in order to rid them of sugars which, coming from the broken cells, might lodge in the other tissues and thereby give a false impression as to which tissues actually contain sugars.

*Microtechnique.*—The woody sections of pear and *Robinia* for permanent mounts were prepared according to the schedule given by Chamberlain,<sup>4</sup> using the paraffin method. It was necessary first to desilicify the wood tissue by immersing it in hydrofluoric acid (commercial strength) for several weeks.

In staining, safranin in combination with Delafield's haematoxylin gave very satisfactory results. Safranin or fuchsin with light-green, furnished good differentiation of xylem and phloem tissue.

*Ringing with the Aid of Chemicals.*—An effort was made to ring shoots without injuring the underlying xylem tissue. To do this it was necessary to prevent both mechanical injury and also drying effects.

Potassium hydroxide and zinc iodide, in varying percentages, were painted on the shoots in the form of a ring. In some instances the epidermis was gently scraped away before applying the solutions. In other cases the entire cortical parenchyma was cut away down to the pericyclic fibers, leaving only a thin layer of tissues surrounding the woody cylinder.

By microscopical examination of cross and longitudinal sections through the ringed areas, it was a fairly simple matter to determine if the phloem had been killed and the xylem uninjured.

*Nutrient Solutions.*—Dormant cuttings, approximately fifteen inches long, were taken from the middle of pear shoots in order to secure a length of stem which was fairly uniform in nutritive condition. Sets of twenty such cuttings were placed in solutions of sodium nitrate (0.5 per cent), sodium sulphate (0.5 per cent), glucose (0.2 molar) and distilled water. The cuttings were left in the solutions for two weeks and then transferred to distilled water. Where glucose was used, the solutions were renewed every other day.

In another experiment, solutions of sodium nitrate, glucose, and a combination of nitrate and glucose were forced into cuttings which were then immediately placed in distilled water.

## PRESENTATION OF DATA

The results of the investigation are presented under the following heads: Growth Responses, Starch Deposition and Depletion, The Presence of Sugars, The Effect of Ringing and Bending on Water Conduction, The Sieve-Tubes of the Pear Shoot, Permeability of the Tracheal Cross Walls, Injury to the Xylem Due to Ringing, Observations on *Robinia pseudacacia*, Ringing with the Aid of Potassium Hydroxide, and The Effect of Nutrient Solutions on Bud Growth.

### GROWTH RESPONSES

*Bending.*—About three weeks after the time of bending (July 9), many of the shoots produced secondary lateral shoots from the buds just below the bend. At the time of bending the shoots were still in active growth. Consequently, there was a geotropic response of the perceptive zone and growth again continued upward. This caused a second bend which seems to have had the same effect as a forced bend in producing lateral branches (pl. 1, fig. 1).

Perhaps laterals develop at or behind the bend because of some interference with the passage of food. Such an interference would necessarily be in the phloem because as a later experiment showed, bending does not interfere with the passage of materials through the tracheae.

*Defoliation.*—Defoliation of the upper halves of shoots on July 7 and 10 resulted within three weeks in the development of lateral branches from two or three distal buds. This was true almost without exception. Defoliation at later dates, July 28 and August 6, produced no such results. The growing period may have passed or perhaps defoliation at that date did not as radically disturb the nutritive condition as defoliation in the early part of July.

Harvey<sup>12</sup> found that the ratio of carbohydrates to nitrogen was reduced by defoliation, not only through the reduction of carbohydrates, but also through actual increase in the nitrogen content. This may be responsible for the pushing of the buds following defoliation.

The growth of buds the following spring on the defoliated parts was apparently like that on untreated shoots. The effect on bud



growth of defoliating half of a shoot did not carry over to the following growing season.

*Ringling*.—All ringling done before the last of July resulted in growth of the buds immediately below the ring (pl. 1, fig. 2). This was true regardless of whether or not defoliation accompanied the ringling, although the effect was most marked when accompanied by defoliation. Ringling after August 1 had no effect on bud growth until the following spring, at which time the buds below the ring developed into lateral branches.

*Heading-Back*.—The effects of heading-back are well known. The shoots cut back on July 20 produced growth from a few buds at the end, while those cut back on July 31, just eleven days later, remained almost dormant (pl. 2, fig. 1).

The date marking the end of the season within which buds of the Bartlett pear can develop seems to be well defined. That date was approximately August 1 at Davis, California, in 1923. The shoots cut back on July 20 and 31 show two very definite responses. There were no exceptions in either class.

Perhaps buds remain dormant after August 1 because of nutritive conditions. Hartwell<sup>11</sup> working with *Solanum* (potato), found that a retardation of growth in the plants was always accompanied by an excessive accumulation of starch in the tissues. The decrease of demand for starch, due to retardation of growth, might lead to this accumulation. However, he suggests that the retardation and cessation of growth may be due to carbohydrate congestion rather than that the accumulation is the result of growth retardation.

#### STARCH DEPOSITION AND DEPLETION

During the growth of pear shoots virtually no starch is present in the tissues except in the endodermis, which lies just outside of the pericyclic fibers and which always shows the presence of starch.

Almost immediately upon the cessation of length growth, starch deposition begins. It starts in the upper end of the shoot and continues progressively downward. It appears first, in appreciable quantity, in the medullary rays. The ray cells nearest the bark are filled first and then those inward toward the pith. Starch forms next in the pith and wood parenchyma cells and lastly in the bark, the cortical parenchyma cells being the chief storage tissue of this region.



The fact that the ray cells nearest the bark are the first to contain starch may favor the theory that conduction of carbohydrates is through the sieve-tubes, because these ray cells are the first ones available for storage of materials coming from that direction.

In the spring as growth begins, starch disappears first from the tip of the shoot and then progressively downward. This is the same order in which it is deposited during the summer. In the various tissues, however, it is removed in almost the reverse order from that in which it was deposited. The bark is emptied of its starch, first, next the medullary rays, the wood parenchyma cells, and lastly the pith. Neither the deposition nor the removal of starch follows this order sharply, for the action starts in one tissue before it is entirely completed in another. This indicates in general the order of deposition and depletion.

Sinnott<sup>23</sup> states that starch reduction in the wood takes place first and most extensively immediately around the tracheae. He suggests that this may not indicate carbohydrate conduction through these vessels but rather that the enzymes which convert the starch into a conductive form may themselves be carried in the water stream and hence come first into contact with the starch in the tissues surrounding the tracheae.

### THE PRESENCE OF SUGARS

To determine the exact tissues which contain sugars is a difficult matter. If carbohydrates enter and withdraw from cells in the form of sugar, it is obvious that a sugar test should disclose its presence in practically all tissues. It cannot be concluded, therefore, that because a tissue contains sugar, it functions in the conduction of carbohydrates. However, both Mangham<sup>16</sup> and Czapek<sup>8</sup> report the finding of sugars in the sieve-tubes.

Numerous investigators believe that they have found sugars in the tracheae. From the tests for sugars, using Flückiger's reaction, the writer cannot conclude that the tracheae of Bartlett pear shoots contain these substances. In the preparation of a section for examination many cells are ruptured and their sugar content scattered over the whole section. It lodges in the irregularities of the tracheal walls and is not easily removed even by washing in distilled water. This gives a false impression as to what tissues actually contain sugars.

Nevertheless, in a general way, sugars can be located. They are most abundant in the cortical parenchyma cells. The wood parenchyma, the medullary rays, and even the pith contain appreciable amounts. The greatest amount, however, is in the bark. Proebsting<sup>21</sup> found that the bark of apple shoots contains from two to five times as much total and reducing sugars as the wood does.

#### THE EFFECT OF RINGING AND BENDING ON WATER CONDUCTION

Curtis<sup>6</sup> suggests that the inhibition of shoot growth at nodes below the terminal may be due to the inability of the lower buds to compete successfully for water.

To determine whether ringing or bending interferes with the passage of water and perhaps thereby causes the buds to grow just below the ring or the bend, the stems were subjected to pressure in the way already described, using the apparatus shown in plate 2, figure 2.

The normal relation between three consecutive sections of an untreated shoot was first established (table 1). The capacity is expressed in the number of minutes and seconds required for two cubic centimeters of water to pass through the sections. The sections requiring the shortest time for the passage of two cubic centimeters of water show the largest conduction capacity. The time was taken with a stopwatch. Where three consecutive sections were compared they were all of equal length. The length of sections was about 12 centimeters, except in the case of bent shoots, where a longer section had to be employed to include the bend.

TABLE 1  
THE CONDUCTION CAPACITY OF NORMAL STEMS

Stem No.	Upper Section		Middle Section		Lower Section	
	<i>Minutes</i>	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>
1	11	20	9	50	8	20
2	6	50	5	55	5	42
3	17	25	15	00	12	40
4	8	33	7	50	7	5
5	12	24	11	10	9	55

It is readily seen that the conduction capacity increases fairly regularly from tip to base, a circumstance which is due in all probability, to the greater diameter of the lower portion. This gives a basis for comparison in the tables that follow.

TABLE 2  
THE CONDUCTION CAPACITY OF RINGED STEMS

Stem No.	Upper Section		Middle Section (Ringed)		Lower Section	
	<i>Minutes</i>	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>
1	5	35	13	00	12	15
2	6	21	12	26	7	58
3	3	24	6	29	4	5
4	5	55	12	6	8	36
5	6	14	10	25	6	18
6	8	6	8	50	4	30
7	10	45	14	40	6	10
8	11	55	12	17	6	47
9	9	34	12	5	7	27
10	8	15	9	45	7	50

Ringling evidently decreases the conduction capacity of stems, in some cases as much as 45 per cent. In the first five examples the upper section shows greater conduction than the lower. This is due to the fact that the stem grew considerably more in diameter above the ring than below it. In the last five shoots the ring had completely grown over and the new xylem which had formed had in part overcome the inability of the ringed area to conduct water (pl. 5, fig. 1).

These results seem to favor the theory that water may be the cause of the buds developing below the ring. The following table shows, however, that in the case of bent shoots, something other than water causes the buds to develop.

TABLE 3  
THE CONDUCTION CAPACITY OF BENT SHOOTS

Stem No.	Upper Section		Middle (Bent) Section		Lower Section	
	<i>Minutes</i>	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>	<i>Minutes</i>	<i>Seconds</i>
1	6	37	4	50	4	20
2	8	00	7	12	3	50
3	6	12	4	39	3	50
4	6	55	4	37	3	28
5	10	40	6	33	4	39
6	5	24	3	45	2	56
7	4	38	2	8	2	29
8	25	43	20	50	15	10

(In this table the section containing the bend corresponds to the one containing the ring in table 2.)



The bent section shows no tendency to obstruct the passage of water. This does not favor the theory that water is the factor which causes the buds in the region of the bend to grow; nor that any substance which passes through the xylem is the cause. If we assume that buds grow as a result of a certain internal condition, it would appear that water is not the essential factor. The results indicate that we should look to the phloem rather than to the xylem tissue for an explanation of bud growth.

### THE SIEVE-TUBES OF THE PEAR SHOOT

The sieve-tubes of one-year-old pear shoots lie in a rather well defined zone just within the ring of pericyclic fibers (pl. 6). They are, by virtue of their shape and structure, well adapted for conduction. Cross walls are few because of the great length of the tubes. It is difficult to determine their exact length on account of the frequent intersections of the tubes with medullary rays which extend out into the phloem. Their connections with the medullary rays suggest that important materials are conveyed by the tubes.

Palladin<sup>19</sup> states that a peculiarity of the movement of organic substances is that it is regulated exclusively by the activity of living cells and is the result of this activity. In other words he believes that this movement is controlled by internal conditions, while the movement of water from the soil, which admittedly goes on in the tracheae, is largely controlled by external conditions, such as light, humidity and wind. This fact along with certain anatomical features, he considers good reason to believe that the sieve-tubes, which are live tissues, rather than the dead tracheal tubes, are used for the conduction of foods.

The sieve-tubes of the pear shoot are without sieve-plates in the end walls. The cross walls are thin and plain without pits or pores. But in the side walls of the sieve-tubes there are numerous highly developed plates with large pores. Some writers term these plates "lattices." The fact that the end walls do not contain sieve-plates by no means indicates that the tubes are not adapted to longitudinal conduction. The sieve-tubes lie in a rather definite zone and overlap each other, thus enabling conduction to take place from one tube to another through the lattices much more rapidly than if there were sieve-plates only in the end walls, because there is more complete

contact by this arrangement than by an end contact of very narrow cells like sieve-tubes (pl. 4).

An attempt was made to determine whether bending injures or compresses the sieve-tubes, and thus causes an accumulation of food materials behind the bend, which results in the growth of those buds into laterals. Bark from the inside and outside of the bend was sectioned longitudinally, using the paraffin method, and compared with bark from above and below the bend. No significant difference could be observed between bent and unbent sections either in the condition of the sieve-tubes themselves or in their number. This does not necessarily mean that the tubes were not compressed or otherwise distorted when the bark was still on the bent shoots. The removal of the bark for sectioning probably released any tension present and the tissues might easily have resumed their normal condition.

#### PERMEABILITY OF THE TRACHEAL CROSS WALLS

The permeability of the tracheae to sugars and nitrogenous compounds was tested to determine the possibility of this tissue being a normal channel for these substances. The same method was used as in forcing water through stems to test their conduction capacity. The same shoots used in conduction capacity were tested in this experiment, thereby furnishing a comparison with the results obtained in using water. Glucose, a 2 per cent solution, and asparagin, a 0.4 per cent solution, were used as representing the most common transitive forms of carbohydrates and organic nitrogen, respectively.

The xylem allowed both glucose and asparagin to pass through. The solution of asparagin passed through the stems at the same rate as distilled water, but glucose moved more slowly, probably on account of its viscosity. The same comparative rate between ringed and unringed stems was found as when tested for water conduction.

In all cases the solution was tested for asparagin or sugar after having passed through the stem. This test was a qualitative one to detect if these substances were passing through the xylem tissue or were being withheld by cell walls or membranes. Distilled water gave a negative test for these substances after passing through the stem. This may indicate that normally there is little or no sugar or asparagin within the tracheae, for such material would be washed out by the water forced through the stem and would be detected by the qualitative tests.



Flood<sup>10</sup> showed that the liquid which issues from the hydathodes of *Colocasia antiquorum* does not contain sugars or other organic solutes and also that there is no filtering mechanism within the hydathode which might relieve it of such solutes. This, however, does not necessarily indicate that organic material was not carried in that xylem sap. Priestly and Armstead<sup>20</sup> forced sugar solutions of known concentration through stems and showed that tissues could remove sugar from the solution as it passed through. This action, they state, is reversible; that is, the solution may remove sugars from the stem. Whether it adds or removes sugars is dependent upon the relative concentrations of the tracheal sap and of the solution.

The tracheae of the pear are very small. They average approximately 0.1 mm. in length and not more than 0.02 mm. in width. The length of stem used in these forcing experiments was at least 10 cm. and often much longer. If each trachea was 0.1 mm. long, in a stem 10 cm. in length there would be 1000 cross walls to be passed by materials being forced through a single tracheal tube.

To determine if wood older than the current season's growth would permit glucose and asparagin to pass, these same experiments were repeated with *Eucalyptus* and *Prunus* (apricot and plum). The young xylem of three-year-old branches was cut away, leaving the two and three-year-old portion. This older xylem also allowed these substances to pass when subjected to pressure. Permeability of the tissues does not seem to be a factor which might allow the young xylem to be active in the conduction of organic materials while the older tissue is perhaps used only for water, as Dixon<sup>9</sup> suggests.

Branches of *Pinus* (pine), which has no tracheae but does have a corresponding conductive tissue in the tracheids, were tested by the same methods and were found to allow both glucose and asparagin to pass.

While these experiments do not indicate anything concerning the normal channels of these substances within the plant, they do show that as far as permeability of the cross walls is concerned, there is no objection to the theory that organic materials are conducted through xylem tissue as old as three years—unless it be true that the permeability is affected by cutting the shoots from the tree.



## INJURY TO THE XYLEM DUE TO RINGING

Ringings, plus defoliation above the ring, prevented any starch deposition in the part defoliated; whereas defoliation alone of the upper part of the shoot did not prevent starch deposition. This shows that ringing interrupts the upward movement of carbohydrates. From such experiments as these, Curtis<sup>6</sup> concluded that carbohydrates move exclusively in the phloem. He found that ringing interrupts not only the carbohydrate movement but also, to some extent, the movement of nitrogen.<sup>7</sup>

The objection to ringing, as a method of determining the tissue involved in food conduction, is that the outer xylem is unavoidably injured in the process. It may, therefore, be fallacious to conclude from the fact that ringing interrupts carbohydrate conduction, that carbohydrates move only in the phloem.

Since the outer xylem is injured by the process of ringing, it is important to know the extent of the injury. Longitudinal microtome sections through the ringed area show actual severing of the tissues by the knife used in ringing in addition to drying effects from exposure to the air. The tracheae appear to be clogged with materials and callus plugs for some distance into the wood past the extent of the knife injury (pl. 5, fig. 1). Measurements with an eyepiece micrometer showed that from 20 to 25 per cent of the xylem cross-section of one-year-old shoots was rendered non-functional for conduction as the result of ringing.

The inner xylem, however, is uninjured by ringing and is available for conduction. The continued growth of a shoot after ringing indicates that water must move through the inner xylem. The vessels of the pear do not develop tyloses as a result of ringing. To make certain that the technique used for determining the presence of tyloses was effective, the method was applied to *Robinia*, the classic example of a plant which naturally produces these structures within the tracheae.

If the xylem tissue is normally used for carbohydrate conduction, why should not all of the xylem in a current season's shoot be available for such a function? In that event, injury to the outer xylem alone due to ringing should not completely prevent this movement, as it apparently does.

Some of the rings grew over, the tissue regenerating from above and below. When this had taken place the growth of buds below the ring ceased, leaving short laterals of varying lengths. Moreover, the carbohydrates were again able to pass, which resulted in a good deposition of starch above the ring. It could not be determined whether the laterals stopped growth because the carbohydrates (and perhaps nitrogenous materials) again flowed by to the upper part of the stem or whether the natural end of the growing period, whatever its cause, had been reached. The fact remains that the growing over of the ring, the cessation of bud growth below this area, and the resumption of upward carbohydrate movement all occurred simultaneously.

Because carbohydrates again moved upwards after the ringed area had regenerated new tissue, it was first thought that this new movement must be through the phloem. However, longitudinal sections through the ringed area showed that both new phloem and new xylem had bridged the gap and that both sieve-tubes and tracheal tubes anastomosed through this region (pl. 5, fig. 1). This would indicate that carbohydrate conduction is restricted to the outer xylem or to the phloem or to both.

#### OBSERVATIONS ON *ROBINIA PSEUDACACIA*

This species was included in the discussion because it was found to exhibit features which contribute to the evidence on food conduction. Five and six-year-old branches were sectioned and tyloses were found to have completely clogged, without exception, every tracheal tube over a year old. Only in the outermost xylem the current season's growth—could tracheae be found free from obstruction. Not all of the vessels even of this new wood were open, but there were enough unobstructed tracheae to form a very definite area for conduction (pl. 3).

The efficacy of these tyloses as plugs was tested by attempting to force water through the stems. When the whole cross-section of stem was intact, water and also glucose and asparagin were easily passed through the tracheae. However, when the outer ring of unobstructed tracheae was cut away, leaving only the tylosis-plugged vessels, not even distilled water could be forced through.



Since tyloses so effectively block the tracheal tubes, this is further evidence that tyloses are not formed in the ringed or bent pear shoots, for water could be easily forced through those stems, even with the outer xylem cut away.

The formation and depletion of starch reserves in *Robinia* furnish interesting observational evidence as to the tissue involved. According to Atkin's<sup>1</sup> theory, carbohydrates are conducted into the xylem from the bark by the medullary rays and are stored in the wood parenchyma cells which form a sheath of starch-containing elements around the vessels. In the spring the carbohydrates in these parenchyma cells diffuse into the tracheae which they border and upward translocation through the tracheae takes place.

In *Robinia* the sheath of starch-containing elements around the vessels is marked, but as has been said, the vessels themselves are entirely blocked by tyloses. The carbohydrates, therefore, could neither enter the wood parenchyma cells nor leave them by means of the adjacent tracheae. Yet this parenchyma tissue is filled and emptied of its starch each year. In this species the path of the carbohydrates stored in the wood parenchyma must pass through the medullary rays to the phloem or to the very outermost xylem.

#### RINGING WITH THE AID OF POTASSIUM HYDROXIDE

The applications of zinc iodide to the shoots proved unsatisfactory as a method of killing a band of phloem. Wherever it was used it was carried upward in the bark and killed the shoot above the point of application. Strangely enough, the portion of the shoot below the point of application was always uninjured. It appears that movement took place only in an upward direction. The solutions were applied in the spring of the year.

Potassium hydroxide (7 per cent solution), when applied to areas where the cortical parenchyma had been cut away, killed the phloem in most instances, without perceptible injury to the xylem. It did not travel in the bark, as did zinc iodide, but remained in the tissue where it was applied.

This method of cutting away the cortical parenchyma before applying the solution of potassium hydroxide was the only one which gave the desired results. Merely scraping away the epidermis did not permit the potassium hydroxide to penetrate sufficiently far to kill



the phloem elements. Cutting away the outer bark without applying potassium hydroxide was unsuccessful because new phloem was developed from the cambium to replace that which drying might have caused to become non-functional. Where potassium hydroxide was used, this difficulty was overcome by frequent applications of the solution in order to kill any newly differentiating phloem elements. That the phloem was killed by this method was determined by microscopical examination. The sieve-tubes and their associated cells had collapsed and become discolored and seemed to adhere to the xylem in the form of a dry, protective covering (pl. 7).

Shoots were ringed in two places by this method and the buds removed between the rings in order that they might not use food in growth. As the shoots started to grow, the starch in the tissues between the two rings was not withdrawn to satisfy the demands of the growing regions above and below. Yet the xylem tissue which was whole and apparently uninjured was available in its entirety for the conduction of this stored food past the rings. The tissues above and below the ringed area were entirely depleted of their starch reserves shortly after growth had well started.

As a check on this experiment, portions of shoots were disbudded without being ringed. The starch in the disbudded regions was depleted by the growth of the buds above and below. It appears from this experiment that the non-removal of starch from between two rings is probably due to the fact that the xylem does not conduct carbohydrates.

It is of importance to note that this method of ringing with the aid of potassium hydroxide, in which the xylem is not injured, results in the growth of laterals from buds below the lower ring (pl. 5, fig. 2). Ringing, by removal of a band of bark down to the xylem, brings about the same response. Apparently ringing with the aid of potassium hydroxide is a method just as effective.

To say that starch between two rings never disappeared would be incorrect. Radial growth of the stem, which unavoidably takes place, slowly made demands on the stored foods in that locality. However, it required a long period for radial growth to deplete the starch reserves; whereas, in disbudded portions of shoots without ringing, the starch was rapidly withdrawn by bud growth above and below these regions.

Radial growth of shoots begins about the same time as bud growth. The new radial additions to the xylem and phloem can be observed within a few days after the buds start. As the apical buds start activity, radial growth in that region begins but it does not take place at lower regions on the shoot until the lower buds break. It appears that the conditions favorable to bud growth may also be favorable to radial growth.

### THE EFFECT OF NUTRIENT SOLUTIONS ON BUD GROWTH

On February 12, four weeks after starting the experiments with nutrient solutions, the buds at the upper end of all the cuttings started to grow into laterals. At the same time the basal buds of the shoot treated with sodium nitrate started to grow, but basal shoots treated with glucose, sodium sulphate and distilled water remained dormant (pl. 8, fig. 1). The absorption of sodium nitrate by the basal portions of the cuttings probably lowered the value of the carbohydrate-nitrogen ratio by increasing the nitrogen factor. The growth of these basal buds cannot be attributed to proximity to the water for the basal buds of cuttings receiving other treatments did not grow at this time.

Three weeks later (March 2) the lower buds with the other treatments did make a weak growth. An examination of the starch content of the tips and bases of the cuttings showed that in every case the growth of the apical buds had been continued at the expense of carbohydrates in the base. The apical ends of the cuttings were still packed with starch in spite of the fact that the buds at that point had elongated several inches. The bases were generally entirely emptied of starch. This depletion of carbohydrates in the base by demands made by the tip might also lower the carbohydrate-nitrogen ratio.

In the second set of cuttings in which the solutions were forced through the stem and then placed in distilled water, approximately the same results were obtained with glucose and nitrogen as in the first experiment. In order of regenerative activity of the basal buds, the various treatments ranked as follows: sodium nitrate, sodium nitrate plus glucose, distilled water, and glucose. It is interesting to note that glucose alone forced into the stems at the basal end entirely inhibited the growth of the lower buds.



Where the nitrates and glucose were forced in together in equal parts, the ratio was probably lowered despite an increase in carbohydrates. A very little increase in nitrogen may lower the ratio materially. This is evidenced by the effects of relatively small applications of sodium nitrate on the growth and condition of large trees.

Sodium sulphate had no effect in bringing about the growth of basal buds. This would indicate that the response to sodium nitrate was due, not to the sodium, but to the nitrate radical.

Auchter<sup>2</sup> planted dormant privet cuttings so that half of the roots would grow in quartz sand while the other half grew in a rich loam soil. Growth started in the branches directly above the loam soil, while there was no growth on the quartz side until three weeks later. He concluded that the nitrogen in the soil was probably responsible for this early breaking of the rest period. His experiments show also that normally there is little or no crossing over of mineral nutrients or elaborated materials from one side of the plant to the other.

Harvey,<sup>12</sup> in studying the growth of apple shoots with special consideration of the rôle of carbohydrates and nitrogen, found that the tip of the shoot is higher in nitrogen, phloridzin, soluble solids, and water than is the middle or base of the same shoot. The base is higher in carbohydrates. He found that ringed shoots have less nitrogen, phloridzin, and soluble solids above the ring, but more carbohydrates. In other words, the chemical situation was reversed by ringing. Defoliation of a portion of the shoot, either basal or terminal, resulted in an increased nitrogen and a decreased carbohydrate content in that portion.

## DISCUSSION OF RESULTS

Ringing, bending, and notching, and other methods of injuring the bark, produce bud growth immediately below, rather than above the injury. This fact is significant, for it indicates that the response is probably due either to the absence of some substance or stimulus prevented from coming down past the injury or to the accumulation below the injury of some substance moving toward the tip. Because defoliation brings about the growth of buds into laterals, it seems that this growth may be associated with nutritive conditions. Defoliation, it should be expected, would decrease the amount of carbo-



hydrates. Harvey<sup>12</sup> found this was true, and also that the amount of nitrogen in the defoliated portion was increased by this practice. This brought about a lowering of the carbohydrate-nitrogen ratio which may have initiated growth. The carbohydrate-nitrogen theory of Kraus and Kraybill<sup>14</sup> is so susceptible of application to nearly all situations of this kind that it must be used with care. However, it seems to furnish a feasible explanation of the growth response to sodium nitrate.

Although various practices such as defoliation, bending, and ringing caused buds to grow, there was no response to these treatments after a certain period. No treatment after August 1 had any effect on bud growth. Perhaps, after this time, the elaborated materials start to move downward into the lower parts of the tree. More probably there are other factors operating which prevent a response so late in the season. This raises the question—does length growth stop and the terminal bud mature because the movement of organic foods is reversed, or do these foods reverse their direction because growth has ceased and the food is no longer needed to meet the demands of a growing tip? The latter hypothesis is more probable because starch is not deposited in the tip until after length growth has ceased.

Howard<sup>13</sup> is inclined to believe that rest sets in on account of the inhibition of enzyme activity which is due to an over-accumulation of the products of their work. Two factors, the approach of cold weather and the inhibition of enzyme activity by the accumulation of carbohydrates, act together, he states, to compel the shoots to cease length growth and mature the terminal bud. This theory shifts the responsibility for growth cessation to retarded enzyme activity rather than to carbohydrate accumulation and its relation to the nitrogen factor from a nutritive standpoint.

The fact that stimuli bring about growth responses in buds only within the period of the growing season, would suggest that perhaps the matter of dominance is related to the rest period which may, in turn, be dependent upon the nutritive condition as a result of carbohydrate-nitrogen relations. There is, however, a distinction to be drawn between the breaking of the rest period and the growth of the upper buds into laterals. The buds on the lower part of the shoot come out of the rest period and form a leaf or a cluster of leaves but normally do not elongate into laterals. There appear to be two factors,

or groups of factors, operating; one causing the buds to come out of the dormant condition, the other causing them to elongate into laterals. It is, nevertheless, not impossible that both of these responses are the results of nutritive conditions.

Since fruit buds are produced chiefly at the terminal ends of pear shoots (pl. 8, fig. 2), it is apparent that pruning, and especially heading-back, not only seriously decreases the bearing possibilities of the tree but also discards much of the growth made by the tree at the expense of food and energy. Perhaps pruning as it is commonly done, is an extravagant and wasteful practice. Bending, with a little judicious pruning, may prove to be a better orchard practice for pears. The food is thus diverted for the production of numerous fruiting laterals rather than for lengthy vegetative growth which must be cut back in order to keep the tree within a manageable height.

The appearance of fruit buds at the apical ends of the shoots seemed at first contradictory to the theory that buds near the apex form lateral branches as a result of a growth-favorable carbohydrate-nitrogen ratio. It appeared that there were two responses at the terminal portion of the shoot, vegetative vigor and fruitfulness, which are supposedly the results of two opposite nutritive conditions. A closer examination of the facts revealed that the two responses are in harmony with the carbohydrate-nitrogen theory. Harvey's<sup>12</sup> analytical results show that in the spring when the growth of buds is initiated, the tip portion of an untreated shoot is relatively high in total nitrogen and low in total sugars. Theoretically, this supplies the proper conditions for the growth of these buds into laterals. As the season progresses, the nitrogen in the tip decreases while the sugars increase until in the last part of June, just at the time when bud differentiation takes place, the sugar content of the tip is at its maximum. This furnishes a satisfactory explanation for the formation of fruit buds at the tip during late June, and also for the growth of laterals in this region at the beginning of the growing period.

The experiments in which solutions of glucose and asparagin were forced through ringed shoots indicate that so far as permeability of the tissues is concerned, there is no objection to the view that carbohydrates and organic nitrogen move through the xylem, unless, as has been pointed out, the permeability of the xylem tissue is altered when the shoots are cut from the tree.



Ringling, it was found, does interfere considerably with the passage of water. This interference is due to actual injury of the xylem tissue in the process of ringling. Were it not for this injury, ringling would be a simple and effective method of determining what tissues are involved in food conduction. One of the forms of injury to the xylem, suggested by Dixon,<sup>9</sup> is the formation of tyloses within the vessels. This work shows that no tyloses were formed in ringed pear shoots, although carbohydrate conduction was interrupted. The injury to the outer xylem elements occurring as a result of ringling was due to actual severing of the outer tissues by the knife, drying from exposure to the air, and clogging of the tracheae with substances deposited in them.

This injury, however, is confined to the outermost xylem, leaving the inner 75 or 80 per cent of the wood uninjured and available for conduction. The question arises, is there any reason to believe that the outer xylem of a current year's shoot may be used for organic materials while the inner portion is used only for water and perhaps minerals? In a branch several years old it may be that only the new xylem is active in conduction, but this distinction can hardly be drawn in the case of shoots of the current season where the xylem is all so newly formed.

The attempts to ring shoots without injuring the outer xylem indicate that conduction of carbohydrates probably take place only in the phloem. Shoots were successfully ringed with the aid of potassium hydroxide and the outer xylem was apparently unaffected, yet this tissue did not serve to move the starch reserve from between the rings.

## SUMMARY

On shoots of the Bartlett pear there is a marked tendency for the apical buds to grow out into lateral branches while those toward the base normally do not elongate.

Ringling, bending, and heading-back of shoots resulted in the growth of buds immediately below the ring, bend, or point of detachment. Any bud could be thrown into growth by these methods. However, no treatment of the shoot after August 1 would induce bud growth.



Defoliation of the terminal portions of shoots before August 1 always resulted in a prompt growth response of the buds in that portion. This fact suggests that nutritive factors are involved in the initiation of bud growth.

This belief was strengthened upon finding that a five-tenths per cent solution of sodium nitrate was effective in bringing about the growth of basal buds of excised shoots. Similar solutions of sodium sulphate had no such effect.

Ringings of shoots was found to interfere to some extent with the passage of water beyond the ring. However, bending, which also results in bud growth, did not interfere in the least with the rate of water passage through the tracheae. This suggests that neither a change in the water supply nor a change in the supply of any substance which might move in the tracheae is the factor which initiates bud growth.

If substances which move through the tracheae are not responsible for the initiation of bud growth, perhaps the responsibility can be laid to foods which move through the sieve-tubes. The investigation from this point, then, is contributory to a determination of the tissues concerned in the conduction of foods.

Starch deposition in shoots of the Bartlett pear begins very shortly after the cessation of length growth. Starch is deposited first at the tip of the shoot and then progressively downward toward the base. As growth begins in the spring, it disappears from the tip and then from the lower regions of the shoot successively. In the various tissues of the current season's growth, starch appears in the following order: medullary rays, pith, wood parenchyma, and bark. It disappears from the tissues as growth begins in the order: bark, medullary rays, wood parenchyma, and pith.

Micro-chemical tests for sugars show that the bark is much higher in sugar content than is the wood. The cortical parenchyma is the tissue abounding most in these carbohydrates.

Solutions of glucose and of asparagin were forced through excised shoots and were found to pass readily. This fact indicates that the tracheal cross-walls are permeable to these substances, and that so far as permeability of the tissues is concerned there is no objection to the belief that these foods move in the xylem.

Ringings of Bartlett pear shoots, by removal of a band of bark from the woody cylinder, interrupts the longitudinal movement of

carbohydrates. The objection to ringing as a method of determining the tissues involved in carbohydrate conduction is that tylosis formation and other factors may prevent the xylem from functioning in conduction.

Tyloses, when present in *Robinia pseudacacia*, effectively block the passage of water and solutions of glucose and asparagin through the tracheal tubes. Observations on tylosis formations indicate that the conduction of foods in this species is limited to the phloem or to the very outermost xylem. The inner xylem is completely obstructed by tyloses.

Ringing did not induce the formation of tyloses within the tracheae of pear shoots, but it did affect the xylem by actual mechanical injury and by clogging and drying of the outer vessels.

However, the inner xylem (75 to 80 per cent of the total) of ringed shoots was uninjured and was available for carbohydrate conduction but evidently was not used for this function, as ringing interrupted carbohydrate movement.

Both the new and the old tracheal tubes of normal excised pear branches allowed water and solutions of glucose and asparagin to pass through under pressure. This was determined by tests made after the removal of the radial growth of successive years. The results indicate that there is little reason to believe that the outer and inner xylem act differentially in the conduction of foods and water, i.e., that the outer xylem conducts only foods, while the whole cross-section is used for water transport. There is still less reason for believing this to be true of shoots of the current season where all of the xylem is so newly formed.

A method is described by which shoots were ringed without perceptible injury to the xylem, thereby overcoming the objection to the usual method of ringing. Even though the outermost xylem was uninjured, carbohydrate movement was interrupted just as though the shoots had been ringed in the usual way.

This study of the conductive tissues in shoots of the bartlett pear seems to indicate that, in this plant, the phloem is the tissue largely concerned in the longitudinal movement of foods and that there is a direct relationship of food movement to dominance of the apical buds, in that the nutritive condition from the carbohydrate-nitrogen standpoint is presumably involved.



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## PLATE 1

Fig. 1. A pear shoot, bent July 10; photographed August 15. The bend near the tip is due to geotropic response but appears to have the same effect on bud growth as the forced bend. Note the lateral growth behind each bend.

Fig. 2. A pear shoot ringed in March. Vigorous growth was produced from a few buds below the ring. Shoots ringed after August 1 did not show this characteristic response until the following spring.

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Fig. 1



Fig. 2





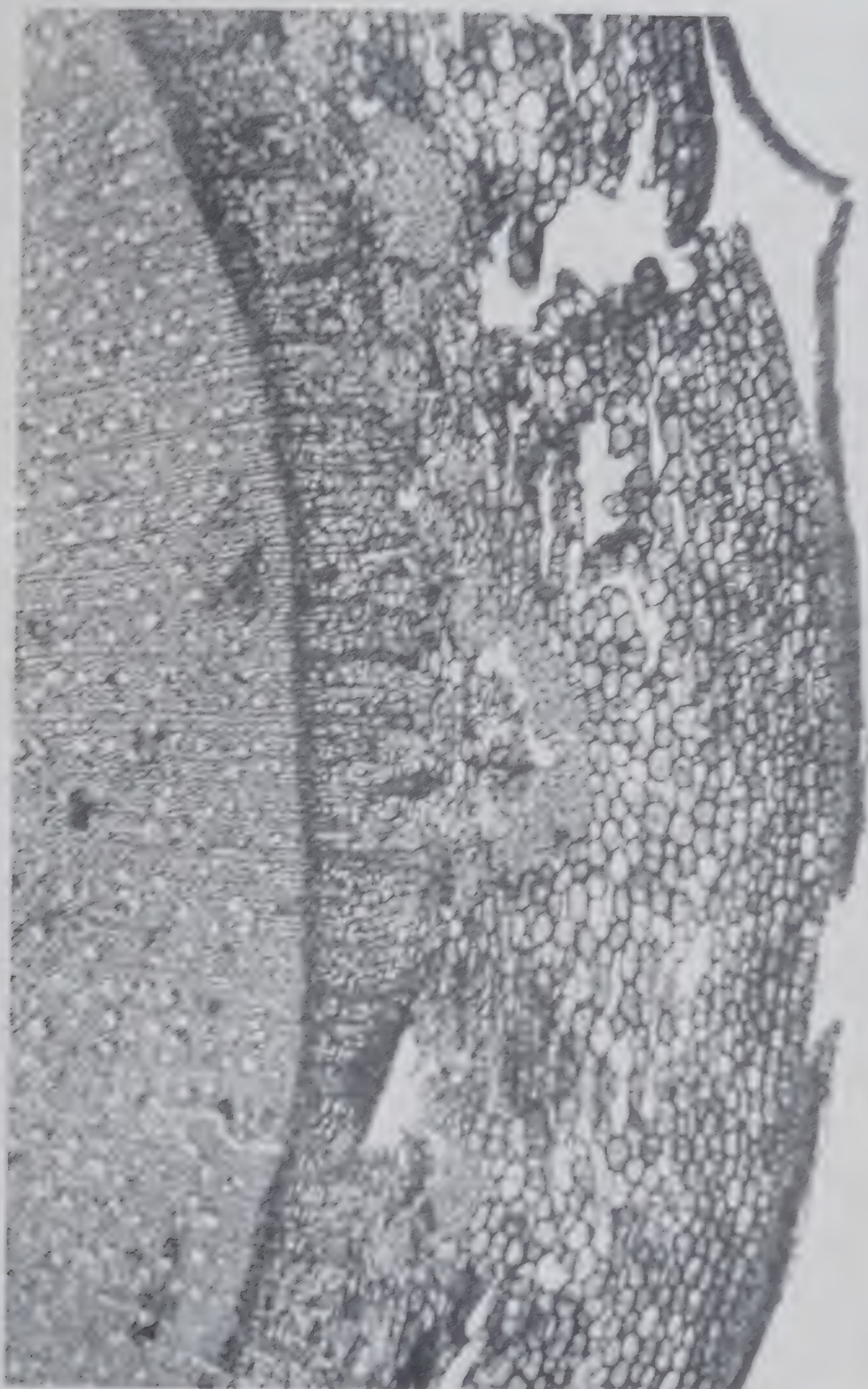


### PLATE 6

A cross-section of a normal pear shoot. The sieve tubes lies in a rather well defined zone between the cambium and the ring of pericyclic fibers. Interruption of carbohydrate movement was accomplished by cutting away the cortical parenchyma (to the pericyclic fibers) and painting the wound with potassium hydroxide in order to kill the phloem elements.

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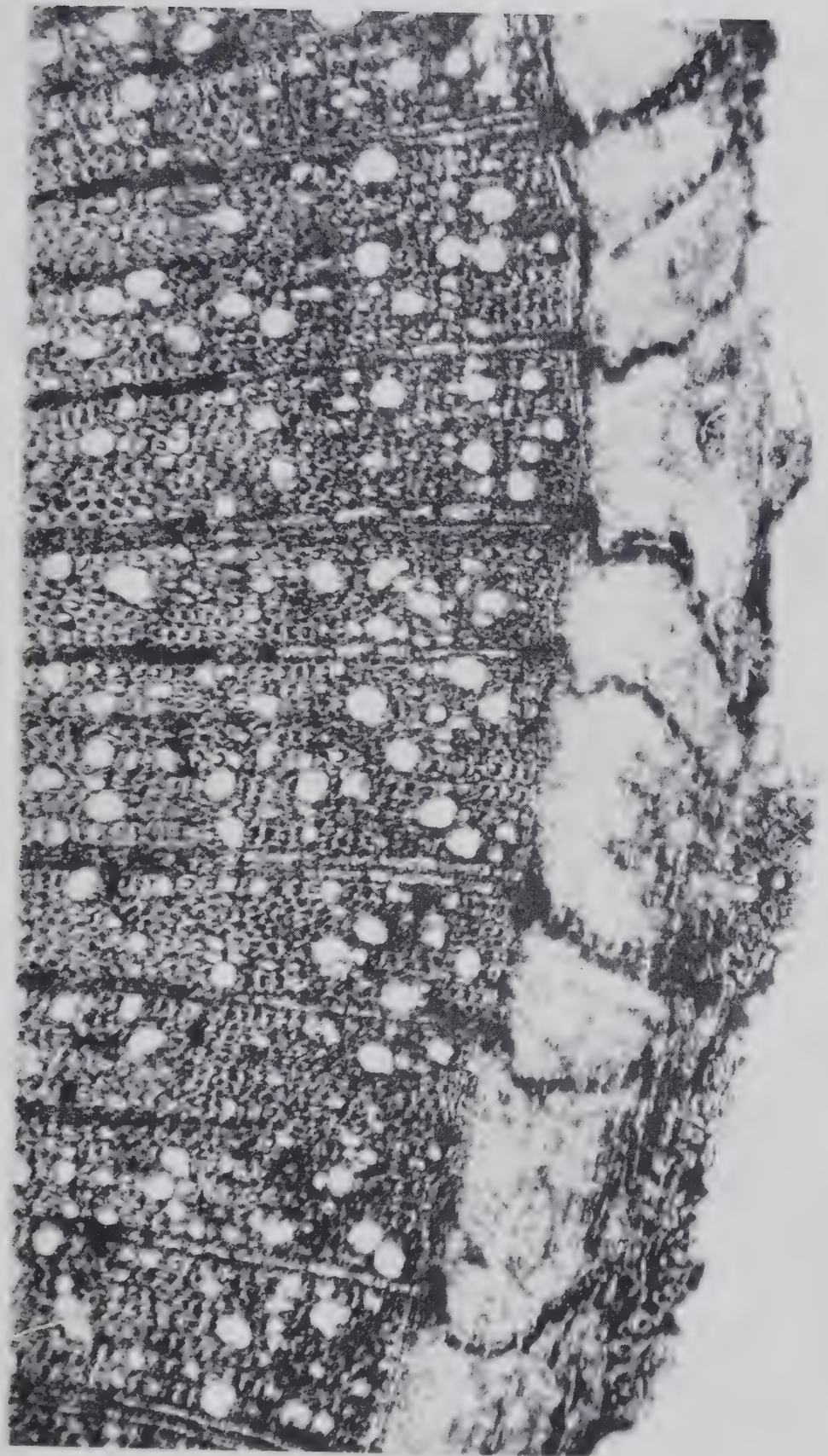


## PLATE 7

A cross-section of a pear shoot ringed in the method described in plate 6. The sieve-tubes are dead, collapsed and discolored—forming a protective covering around the xylem. The xylem itself is uninjured; even its outermost edge appears alive and unaffected by this treatment.

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### PLATE 8

Fig. 1. Pear cuttings which were placed in a one half per cent solution of sodium nitrate. Note that the basal buds are starting to grow. In the solutions of glucose, sodium sulphate and distilled water, the basal buds remained dormant. Since sodium sulphate did not cause the basal buds to grow it would appear that the response to sodium nitrate was due, not to the sodium, but to the nitrate radical.

Fig. 2. Typical shoots of the Bartlett pear, showing the location of flower-buds. It is from this same locus on the shoot that lateral branches are produced. It is interesting to note that buds containing flowers start activity long before the purely leaf-buds on the lower portion of the shoot.





FIG. 1



FIG. 2











## PLATE 2

Fig. 1. Pear shoots headed back on July 20 (left) and July 31 (right), just eleven days apart. This indicates the end of the period within which buds may elongate into lateral branches. Defoliation, bending, ringing, or heading back produced no growth response after August 1, at the University Farm, at Davis.

Fig. 2. The pressure device used to force solutions through the shoots to test their conduction capacity and permeability. The height of the liquid column was four and a half feet. The sections of stems were cut and adjusted to the rubber tubing under water in order to prevent air from entering the tracheae.



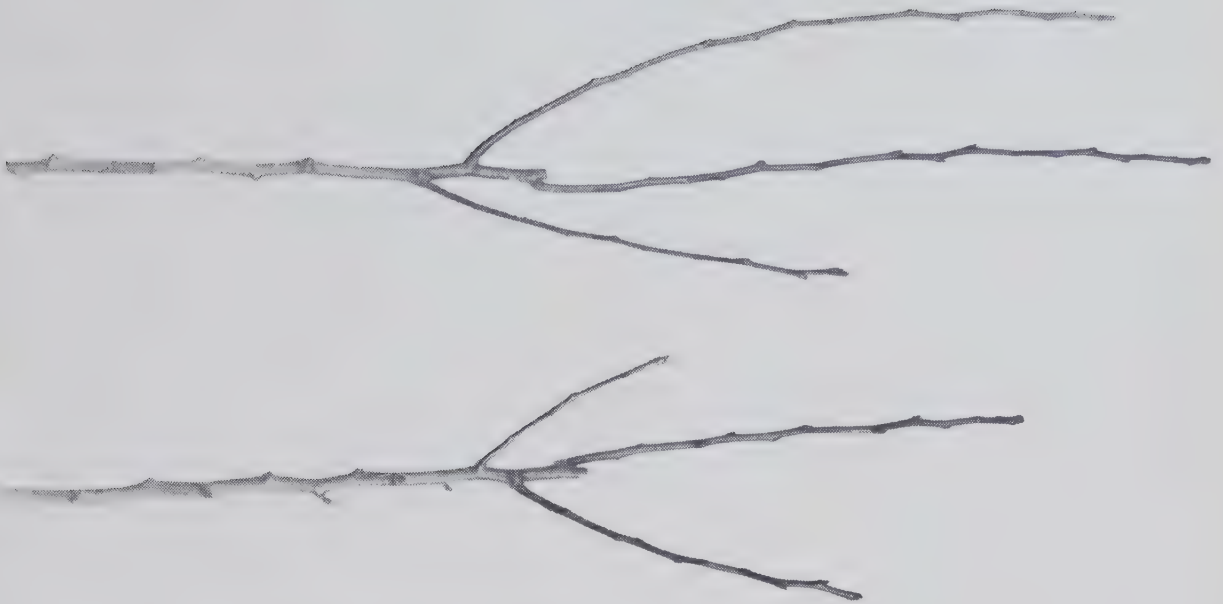


Fig. 1

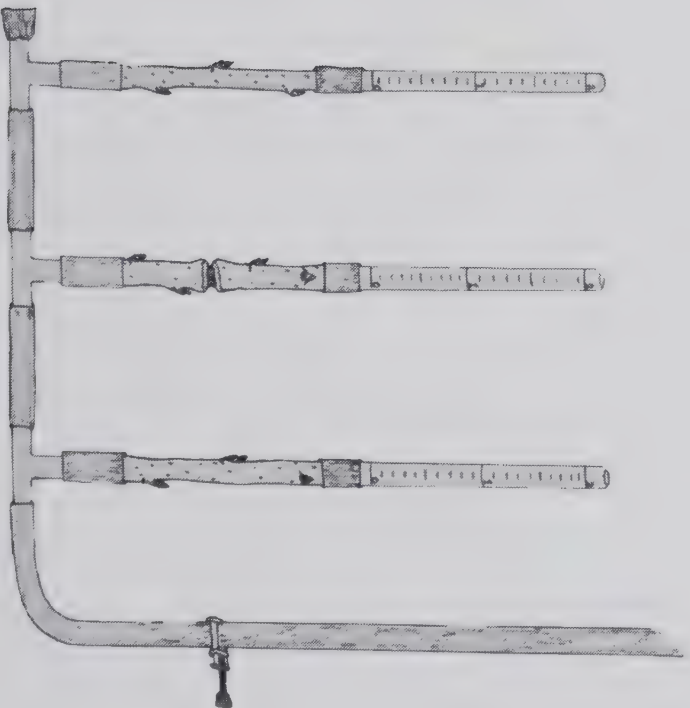
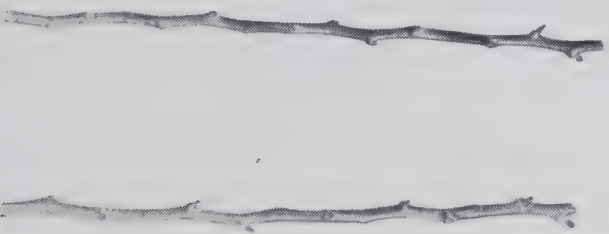
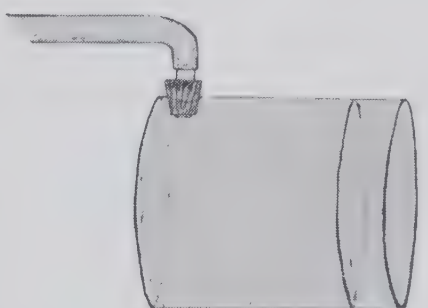


Fig. 2







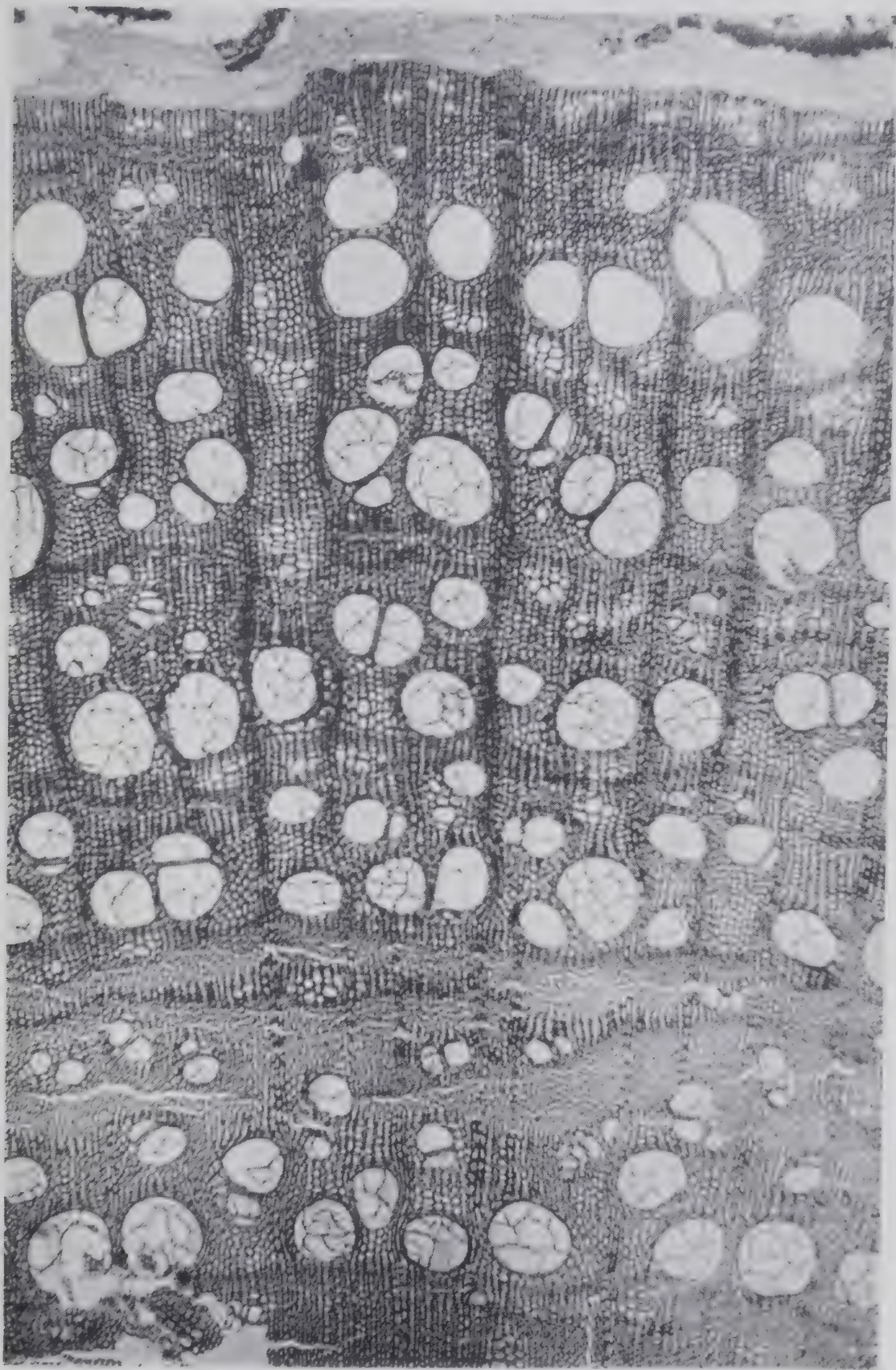


### PLATE 3

A cross-section of a six-year-old stem of *Robina pseudacacia*. Note that tyloses completely block the tracheal tubes except in the current season's growth. These tyloses form an effective barrier to materials which might otherwise move through the tracheae. In this species the conduction of foods is necessarily limited to the phloem of the very outermost xylem.

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#### PLATE 4

A longitudinal section of a pear shoot showing the sieve-tubes. Note their adaptability for conduction—their length and the highly developed “lattices” in their side walls. The medullary ray cells are shown extending into the sieve-tube area.

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## PLATE 5

Fig. 1. A longitudinal section of a pear shoot taken through the ringed area which has completely healed over. Both new phloem and new xylem have been regenerated. Note the injury to the xylem made by the knife in the process of ringing. Note also the clogging of the tracheae in this region while the inner xylem is uninjured and unobstructed. Yet this inner xylem is not used for carbohydrate conduction. The question arises, Is the outer xylem normally used for this function?

Fig. 2. Pear shoots which were ringed in two places by cutting away the cortical parenchyma (leaving the pericyclic fibres) and by applying from time to time a seven per cent solution of potassium hydroxide. The area between the rings was disbudded to prevent the removal of starch by bud growth. The buds below the lower ring are starting to grow.



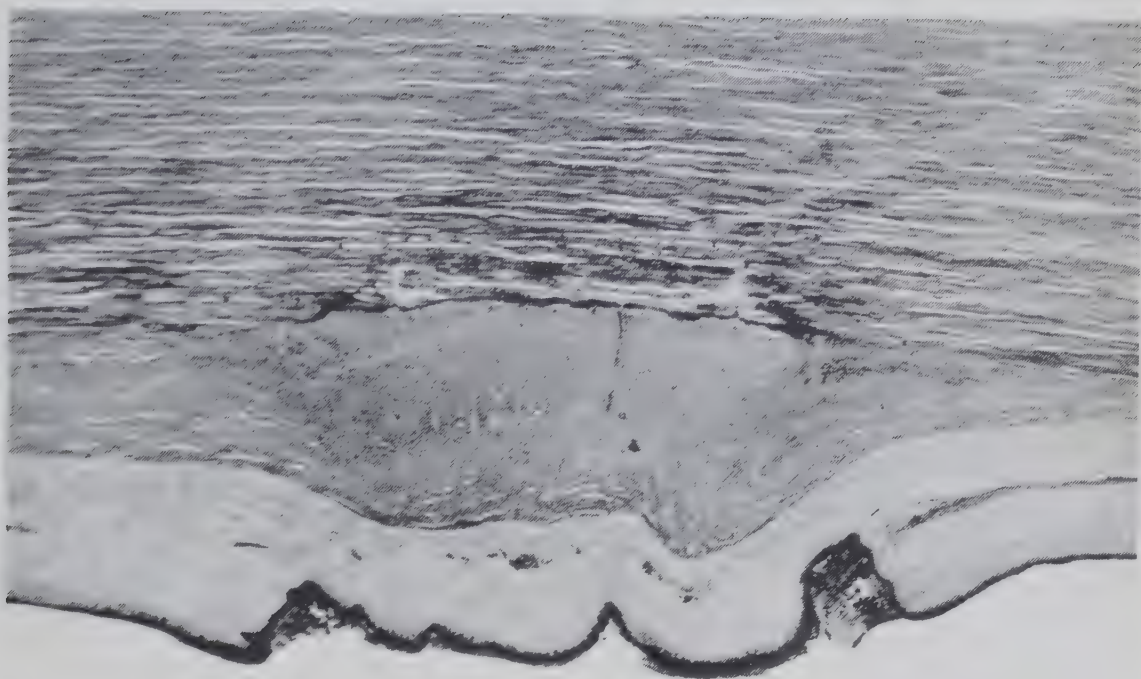
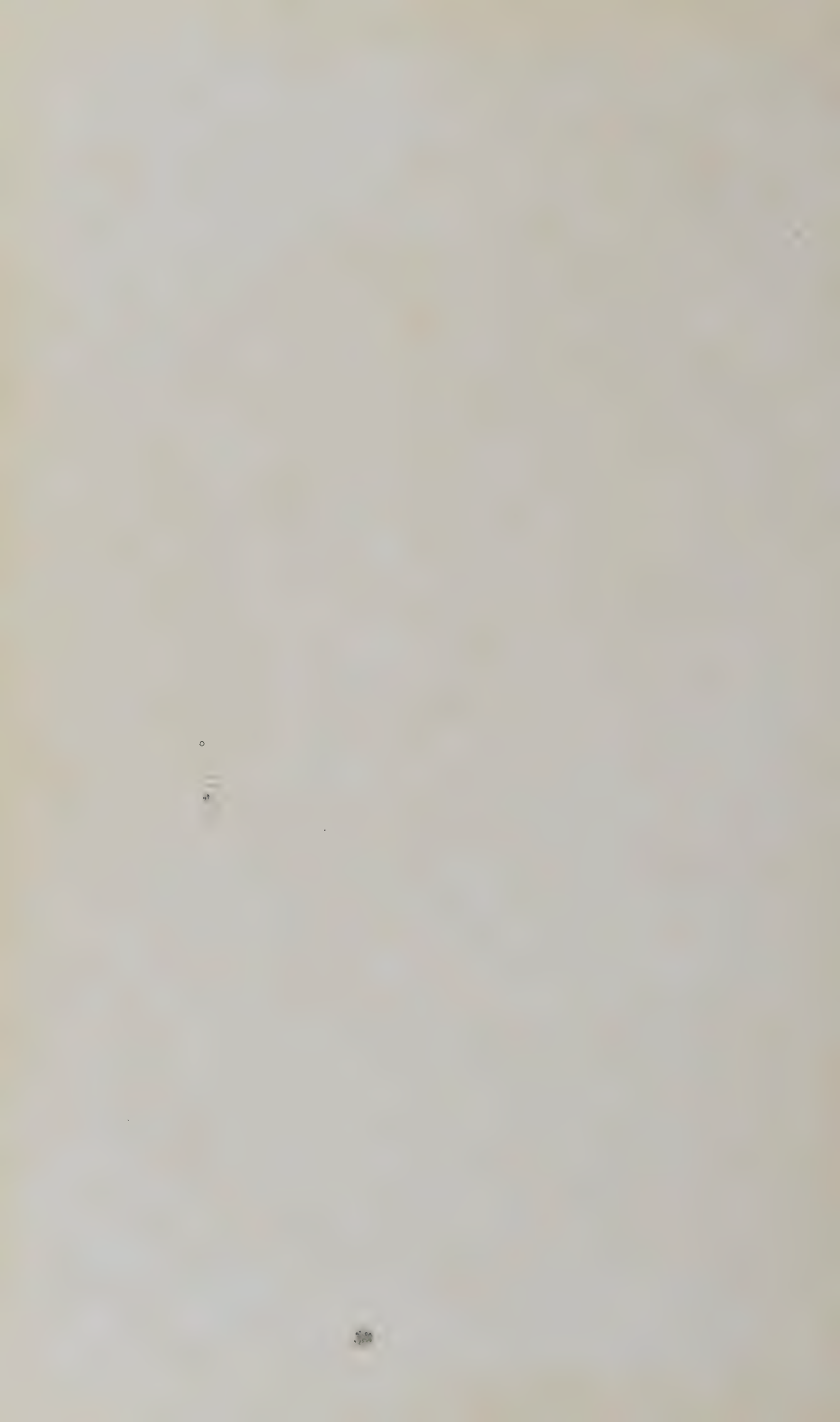


Fig. 1



Fig. 2



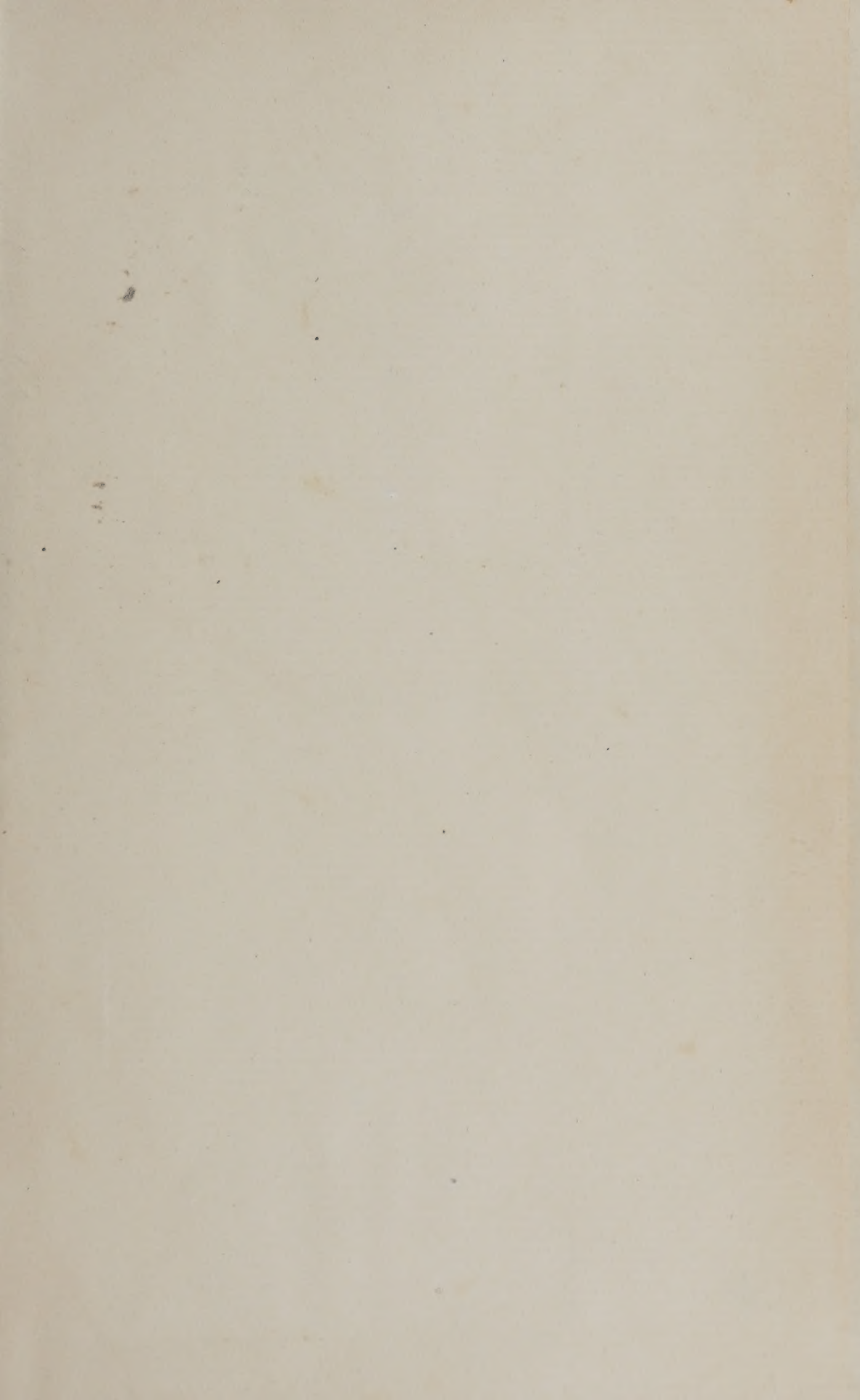


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